

President Reagan and Mr Perle

The US administration seems to be speaking with two voices on arms control. Mr Richard Perle, the hard man from the Pentagon, should be the one to opt for moderation.

THE assumption that statesmen have no time to read the newspapers seems to be gaining ground. That, at least, is the simplest explanation why President Ronald Reagan (at Strasbourg) and Mr Mikhail Gorbachev (in Moscow) used the fortieth anniversary of the ending of the Second World War in Europe to tell their separate audiences of the other's malevolence. Although much of the rhetoric may have been directed at Western Europe in general, and not at the European Parliament (Mr Reagan) or a celebratory parade in Moscow (Mr Gorbachev), both declarations will have helped chiefly to reinforce the belief that nobody is in much of a mind to win agreement at the negotiations of strategic arms soon to resume at Geneva. But if the negotiations are again a failure, people may well remember what the superpower leaders were saying last week.

As when a bridge-player can win only when some vital card is on his left, he had better play on that assumption, so it is prudent to suppose that the rhetoric masks a more benign state of affairs. But then the question arises of the remarks of Mr Richard Perle, assistant secretary at the US Department of Defense, who was explaining to a congressional committee again last week why, in general, arms control agreements favour the Soviet Union rather than the United States, why the Soviet Union can be relied upon to break any agreement that may be signed and why, in particular, the United States need not be too meticulous in observing the spirit of the SALT II treaty, the unratified agreement which limits the numbers of strategic missiles which each side may deploy, and which has been broadly followed since it was signed in 1979. Mr Perle, who knows well enough that other people read the newspapers, has never hidden his belief that arms control is a fruitless pursuit. But making such a song and dance about it is bound to give Soviet readers the impression that it is a part of the US administration's policy, which is presumably untrue.

Mr Perle's views nevertheless deserve attention. There is a sense in which agreements favour the Soviet Union. The US system is open to public scrutiny, much of it relentlessly vigilant, while the Soviet system is, by comparison, secret. So, in principle, the Soviet Union can sign an agreement and then promptly break it. That is what Mr Perle suggests. The snag is that the Pentagon's attempts so far to prove Soviet cheating on arms control agreements are thoroughly unconvincing. Allegations of the proxy use of toxic chemicals in South-East Asia are dubious. The allegations of breaches of the threshold test-ban treaty are only marginally convincing, given the uncertainties of seismic interpretation. The surest transgression so far established is the Soviet

construction of a phased-array radar at Krasnoyarsk in central Siberia, apparently a breach of the anti-ballistic missile treaty of 1972 (which has been ratified). The United States has a perfect right to demand a better explanation than has been given so far. What Mr Perle leaves out of his calculations is the certainty that the Soviet Union, secretive though it may be, would nevertheless be seriously harmed if it were shown unambiguously to have cheated on a solemn agreement.

In reality, the Soviet Union (like the United States) has a creditable record on compliance with agreements on strategic arms, and did not complain that the US underground test that vented radioactivity to the surface was a violation of the oldest (1953) agreement on nuclear weapons. The agreement not to place nuclear weapons ("weapons of mass destruction") in space has been kept. If anything, the Soviet Union has been more meticulous than the United States in observing the provisions of the Non-Proliferation Treaty (1968) that its partners in the civilian trade in nuclear materials would not be allowed to fashion nuclear weapons from them. Not that either of the superpowers would relish the emergence of other nuclear weapons states. The difference between them is merely that the United States has a more vivid interest in the commercial benefits of the trade, which are often then frustrated by US domestic legislation.

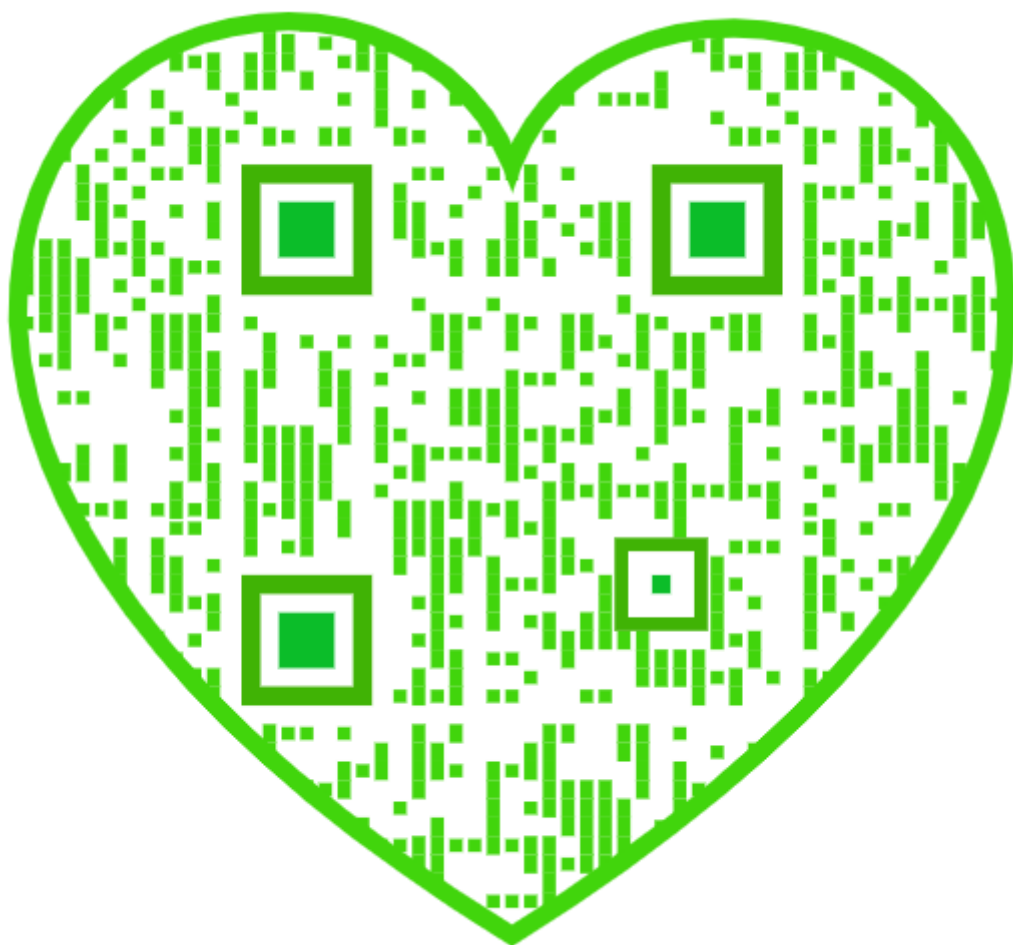
A more accurate version of Mr Perle's conviction that the Soviet Union will always cheat is therefore that the Soviet Union will always honour the letter of its agreements, but will exploit to the full the opportunities it is not formally denied. This, it might be held, is why western Russia was thickly populated with SS20 missiles in the late 1970s, in defiance of the spirit but not the letter of the SALT I treaty (1972), which says nothing about missiles of intermediate range.

Mr Perle now says that the new Soviet missile, called the SS25, which has begun to make its appearance on the ground, is a violation of SALT II because it is an essentially new device, which would be forbidden by the treaty, even though its external dimensions do not differ much from those of the much earlier SS13. Mr Perle's difficulty is that while the treaty allows each side to deploy one new type of long-range missile, the definition of what is new and different is determined (in article IV.8) primarily by the external dimensions (plus or minus 5 per cent). The Soviet Union, which appears to have offered to retire as many old missiles as the new versions it may introduce in the months ahead, may be legally right to claim the SS25 is within the treaty. The issue matters to the United States, which will have to decide in September whether the sea-trials of the new *Trident* submarine will require it to retire an ageing *Polaris* boat. What Mr Perle might gracefully acknowledge is that the faults lie not primarily with the Soviet Union, but in the language of SALT II, which has been overtaken by events in just seven years. Geneva, now, should be partly about the redrafting of this clumsy treaty and partly about its extension to missiles of intermediate range. This should preferably be done before the treaty runs out at the end of December — a deadline specified on the assumption that, by then, there would be more meaningful treaties on the books.

SALT II is therefore not a treaty that people should struggle to see ratified. If that were Mr Perle's case, he would be generally applauded. Where he goes wrong is where he goes further, in saying that the existence of this informal scrap of paper is of no

Biological manuscripts

From the beginning of next week (20 May), Miranda Robertson, Biological Sciences Editor of *Nature*, will be based in the Washington office of *Nature*. Manuscripts offered for publication may be sent, as at present, to either the London or the Washington office; with the benefit of good communications, the speed with which manuscripts are dealt will be independent of the place at which they are received. But authors are asked please in future to send **four copies of manuscripts** intended for publication (one for each office and two for referees). □



significance — that it counts for nothing more than the anti-ballistic missile treaty, now threatened by the star wars programme. The assumption that these documents count for nothing is a psychological mistake. The most striking evidence to the contrary is that the two superpowers, while knowing that the treaties lack the force of law, have nevertheless chosen to behave as if the treaties were binding on them. But to the extent that the states of Western Europe are interested parties, these scraps of paper are the only assurance they have that events will not get out of hand.

It would serve Mr Perle's purpose just as well, and that of the US administration at the same time, if he were to turn the other cheek and challenge the Soviet Union by demanding that the US Congress should ratify the threshold test-ban treaty in its present form, which forbids the testing even underground of nuclear warheads exceeding the equivalent of 150,000 tonnes of TNT. This, broadly speaking, is the practice to which the superpowers have kept since 1972. Congress in its present mood would sign with very little persuasion. (Only the Senate would matter.) The benefits would be that the Soviet Union would be required to produce a stack of seismic data with the help of which suggestions of violations could be more accurately assessed, even retrospectively. If Mr Perle's suspicions are correct, they would be confirmed. If they are not, sweetness and light would spread. Either way, the fear would be dispelled that the US administration is divided on arms control, showing up at Geneva diligently while thinking like Mr Perle. □

Football squalor

The deaths at a British football game should be an invitation to economic realism.

THE British government is in a tragic dilemma over the game of soccer, otherwise association football. Last Saturday, 53 people were killed (with more than a dozen people still missing) when a wooden structure in which several thousand had been sitting caught fire and burned to a cinder in the Yorkshire city of Bradford. But for most of the winter, the government has been worrying not simply about the British economy but about the phenomenon of soccer hooliganism, the way in which bands of football supporters attack each other, or the other side's players, or sometimes the officials who try to ensure fair play. Football crowds have been a worry for British governments ever since 1972, when even more people were killed than last weekend during a stampede at a football ground in Glasgow.

The causes of last weekend's fire and of the long-standing hooliganism of football crowds are linked. British professional football, long since a spectator sport, is by legend the poor man's entertainment. So it was before the invention of the television, since when attendance at football matches has been falling steadily. Now, it is more accurately a poor professional's living. All but the best of football teams scrape by on a shoestring. Their grounds are squalid places. Sitting room is limited and expensive. Most have to stand for a couple of hours at a stretch, often crowded together in a way almost calculated to provoke violence. Yet so parlous is their financial condition that more than a third of the football grounds in England and Wales have been exempted from the safety regulations made law in 1975.

The simple question prompted by the death of 53 people in a tinderbox is whether it makes sense for a British government which has other things to worry about to shelter from economic reality commercial organizations which cannot afford to protect their customers from avoidable risks, let alone provide them with an environment which induces them to watch the game and not attack each other. Whether a country of 50 million people needs more than 100 professional football teams in the top flight (counting those in Scotland) is a matter that should be determined by market forces and not the government. If the outcome were that some were at a loose end on Saturday afternoons, and forced as a consequence actually to play the game they would otherwise be watching, that might be no bad thing. □

Patent rights

Two years late, British academics are to have title to patents arising from inventions.

Two years have passed since the British Prime Minister, Mrs Margaret Thatcher, promised publicly that inventions generated with public support would no longer have to be offered for exploitation to the National Research Development Corporation (NRDC), but only this week has the government decided that the time for changing the present system has actually arrived. From now on, universities will be free to exploit their own inventions, whether or not they arise from research grants awarded from public funds. NRDC will remain in being (as part of what is now called the National Enterprise Board), and may paradoxically be even more ready in the future than in the past to exploit inventions offered by academic researchers, not least because there will be competition. Individual researchers are likely to be excited (and surprised) by the details of the new arrangements, which should provide them with a powerful incentive to market their discoveries, but their institutions will also benefit, on paper at least. So will British academic research now be further transformed by people's ambitions to get rich?

To a first approximation, the answer is no. The new arrangements for the exploitation of British inventions are no different in principle from those in force in the United States since 1972. Before British academic researchers and their institutions start counting their golden geese (to mix two metaphors), they had better reflect on the frequently disappointing experience of US universities, which have often found that the right to the independent exploitation of innovations is often exercised only with difficulty, while the duty to do so (implied by the US legislation) may be onerous. For what many US universities have discovered is largely what NRDC has been saying almost since its foundation in 1947 — that only a tiny proportion of academically-based patents become money-spinners. A large national organization, with a monopoly right of first refusal, can hope to cover the cost of protecting ideas taken onto its books by the proceeds from a few money-spinners. Smaller organizations, even whole universities, are by definition less able to cover their risks.

Even so, it is right that there should be a change in the arrangements in force in Britain. Although NRDC has never managed to demonstrate the touching belief of its founders that Britain was awash with patentable inventions going to waste, and while its work over the decades has been creditable, it has also accumulated a rich fund of ill-will among academic researchers who consider, rightly or wrongly, that their bright ideas have been too often overlooked. The truth, of course, is that no single organization can hope to make consistently wise decisions in such a vast field. The best approach is bound to be one in which different organizations make independent appraisals of novel developments and then agree to back those they fancy with tangible resources, both money and management. With the persistent pressure on university research funds, and the change in general attitudes towards the exploitation of new ideas, ending the corporation's monopoly right will do much more good than harm.

The consequences of this change for the character of what happens in British academic laboratories is unlikely, in the short run, to be profound. To be an acknowledged success as a scientist or engineer will continue to be an important if no longer a paramount consideration. Moreover, NRDC will retain an active interest, and indeed is plainly planning (with government help) to strengthen its links with university researchers. The most likely change is that there will be other venture capitalists scouring the academic laboratories for exploitable innovations, while established companies will have a further incentive to forge academic links, perhaps with particular departments, by offering help with patent protection. The danger, probably not at this stage serious, is that some universities or parts of them may become so immersed in particular relationships that they will cease to be impartial sources of help and advice for industry at large. That is an issue to which university administration should urgently pay attention. □

US-Soviet exchanges

Academy divided over new agreement

Washington

THE US National Academy of Sciences (NAS) is coming under increased criticism for negotiating a renewed scientific exchange agreement with the Soviet Academy of Sciences last January. Details of the agreement, which is still awaiting ratification by the Soviets, are being kept secret by the academy, which has intensified the uneasiness of groups working on behalf of Soviet refusniks and dissidents.

Shortly before the NAS annual meeting last month, a letter, signed by Christian Anfinsen, Paul Flory and Arno Penzias, was sent to all academy members, challenging both the wisdom and the morality of the move to reestablish ties. (The letter was subsequently published in *Science* 228, 530; 1985). Anfinsen *et al.* asked what had changed "to explain the about-face from the moral stance of 1980", when NAS broke off relations with the Soviet Academy in protest at the internal exile of Andrei Sakharov.

The three said Sakharov's condition had in fact worsened, and, citing NAS's own Committee on Human Rights, that there had been many new arrests of scientists. "To have held the negotiating meeting in Moscow", they concluded, "makes it hard to avoid an unfortunate hat-in-hand image."

A sharper challenge has come from Sakharov's son-in-law, Efram Yankelevich, who is living in the United States. After seeing Dr Frank Press, president of NAS, Yankelevich wrote an impassioned letter, taking Press and the academy to task in the strongest terms for having abandoned his father-in-law. The letter has been widely circulated within the scientific community.

Finally, Richard Perle, the hard-line Assistant Secretary of Defense for international security policy, broke government silence on NAS's actions with a strong criticism of his own. Speaking at a press forum arranged by the Scientists Institute for Public Information in Washington, Perle said that he was "disappointed that NAS is plunging ahead" with renewing exchanges. He argued the familiar administration line that the Soviets gain more from such exchanges than does the United

States.

There is little doubt that Press has the support of the NAS membership, but these attacks have put him on the defensive. Press said last week that the differences between him and his critics amounted to a disagreement over tactics for aiding the refusniks and dissidents. He argued that, without face-to-face contacts, NAS's leverage is "non-existent". He said that "sending telegrams that are never even acknowledged may ease people's consciences, but there's no proof they've done any good".

Asked about reports from refusniks that their situation has worsened since he signed the agreement in Moscow (see the letter from Solomon Al'ber in *Nature*, 2 May,

p.10), Press said that he had heard "contrary information" from his sources. And a source familiar with the negotiations said that NAS had demanded considerable concessions, including mention of the Helsinki accord in the text of the protocol. Academy officials have also let it be known that a part of the NAS delegation that went to Moscow met with refusniks there; Press himself did not, however.

As for Perle's criticisms, Press said that "he should have asked me what happened in Moscow before he made those remarks", remarks Press characterized as "intemperate".

Press said that Perle had not taken advantage of the opportunity to be briefed about the agreement (as were State Department officials and the US Ambassador in Moscow).

Press also rejects complaints about the secrecy of the document. He said that it is still in draft form and cannot be made public until negotiations have been completed and the text has been agreed by the Soviets.

Stephen Budiansky

Sakharov

Resignation from Soviet academy

Ottawa

THE Ottawa review conference of human rights performance by the 35 signatory countries of the Helsinki Final Act opened last week to a flurry of rumours that Academician Andrei Sakharov and his wife, Elena Bonner, have received permission to emigrate.



These rumours, which did little to defuse the procedural hassles that delayed the formal opening of the conference by several hours, so far remain unconfirmed.

Almost simultaneously came the news, confirmed by Dr Efram Yankelevich, Mrs Bonner's son-in-law, who acts as Dr Sakharov's personal representative in the West, that Dr Sakharov had announced his intention of resigning from the Academy of Sciences of the USSR, with effect from 10 May, if his conditions of exile are not improved, and if Mrs Bonner is not allowed to travel abroad for medical treatment.

Although the Soviet authorities have, on several occasions, tried to pressurize the academy into expelling Dr Sakharov, these

efforts have been resisted even by party hard-liners in the academy as liable to set a dangerous precedent. Dr Sakharov's voluntary resignation, however, would clearly not be in the interests of the authorities, in view of the considerable comment it would arouse internationally.

Moreover, Dr Sakharov, who will be 64 on 21 May, is past Soviet pensionable age, and the loss of his academy stipend, which he has still been permitted to draw, would not necessarily put him at risk of a charge of "parasitism" (being without visible means of support). Dr Sakharov has previously been reluctant to leave the Soviet Union, feeling that his presence there provides valuable moral support for the human rights movement and for victims of oppression. Hence in 1975, he did not collect his Nobel peace prize in person for fear that he would not be allowed to return to the Soviet Union. Since he was exiled to Gor'kii in January 1980, he has been effectively prevented from giving any assistance to his distressed fellow-citizens, and in 1983 he accepted an invitation from the Norwegian government to settle in Norway — provided, of course, that he is allowed to leave the Soviet Union.

Prospects for the Sakharovs to emigrate are not, however, promising. According to Dr Yankelevich, Mrs Bonner, who was sentenced to three years' internal exile to Gor'kii in August 1984, was excluded from the amnesty proclaimed in honour of the fortieth anniversary of the end in Europe of the Second World War. Dr Sakharov's own exile, of course, is the result of an administrative decision and, technically speaking, has no legal validity.

Vera Rich

Correction

IN the graph illustrating the percentage of approved extramural research grants that are awarded by the National Institutes of Health (*Nature* May 9, p.88), the units on the right-hand axis were incorrectly specified. The range covered should have been from 30 per cent to 60 per cent, not from 3 per cent to 6 per cent. □

Genetic engineering

RAC stakes its claim

Washington

IN the face of proposals to create a new layer of bureaucracy to oversee genetic engineering, the Recombinant DNA Advisory Committee (RAC) of the National Institutes of Health (NIH) has begun to assert itself with a new vigour. At the meeting of the committee on 2 May, members voiced concern that other agencies moving to assert jurisdiction over recombinant DNA — in particular the Environmental Protection Agency (EPA) — lack the expertise that RAC has accumulated in its 10-year existence and that basic research could suffer as a consequence.

The focus of concern is the lengthy proposal of the Office of Science and Technology Policy (OSTP) for the establishment of a "coordinated framework" for regulating biotechnology (see *Nature* 313, 85; 1985). Under that proposal, a Biotechnology Science Board, a sort of "super-RAC", would be created to address generic issues and to settle interagency disputes; in addition, each agency that deals with biotechnology (US Department of Agriculture, National Science Foundation, EPA, Food and Drug Administration and NIH) would have its own RAC to provide advice within the agency.

Although most of this proposed bureaucracy would address only applications for marketing or development of commercial products and would not involve any new statutory authority, EPA has indicated its intention to claim some authority over research. The agency has already said that field tests of microbial pesticides produced by genetic engineering come under the Federal Insecticide, Fungicide, and Rodenticide Act (nothing new), and (a definite departure) that the usual 10-acre exemption would not apply. Under interim rules announced by the agency, anyone who wants to field-test a genetically engineered microbial pesticide on a plot of any size must notify EPA; the agency can then demand a formal application for an "Experimental Use Permit".

EPA also intends to view the DNA in novel microbes as "new chemical substances", subject to the notification and safety-testing requirements of the Toxic Substances Control Act (TSCA). Under that act, EPA must be notified 90 days before the manufacture of such a new substance commences; EPA can demand additional data and with cause can restrict or even ban the product.

In a letter from RAC chairman Robert Mitchell to OSTP, the concern of RAC about the proposal was spelled out; RAC members say that EPA and other agencies appear to be reinventing the wheel, raising questions that RAC was able to lay to rest years ago. And there is strong feeling within RAC that all laboratory research at

least must remain the business of RAC alone.

In discussion at this month's meeting, members made it clear that a major function of the committee since its inception — in the committee's own view, that is — has been protecting basic research from over-regulation, a feat accomplished largely by providing a forum for defusing public concern and heading off demands for regulation *per se* through a demonstration of cautious self-regulation.

Although EPA's TSCA proposal raises the possibility of EPA's regulating even the laboratory research phase of certain commercial biotechnology products, RAC in fact faces little challenge to its claim to having exclusive oversight over laboratory experiments. The major grey area at this point is environmental testing. The RAC staff, still staggering under the paperwork and legal burdens of the Lindow-Panopoulos proposal for field-testing genetically altered ice-nucleating bacteria,

would like to turn it all over to EPA. A proposal by the staff, on which RAC decided to defer action at the last meeting, would allow RAC at its discretion to accept the review of a proposal by other agencies as meeting RAC's requirements. The partial victory by anti-genetic-engineering activist Jeremy Rifkin in the Court of Appeals (see *Nature* 7 March, p.6) has opened the way for continual court challenges of the adequacy of RAC's approval of Lindow's experiment and other environmental-release experiments, another headache that the staff would as soon avoid.

At least a part of the RAC membership, however, is less ready to concede that jurisdiction to EPA. RAC has adopted an informal document to guide those submitting proposals for environmental-release experiments (*Points to Consider for Submissions Involving Testing in the Environment of Microorganisms Derived by Recombinant DNA Techniques*); it was noted at the recent meeting that RAC enjoys the flexibility easily to amend this document with experience; the regulatory agencies face a much more ponderous process.

Stephen Budiansky

US education

More engineers needed

Washington

THE chronic shortage of engineering faculty at US universities has arisen largely because engineering professors are paid \$10-12,000 a year less than their counterparts in industry, according to a \$900,000 study of engineering education* published last week by the National Research Council (NRC). And although the number of engineering PhDs awarded each year will increase over the next few years to 4,000 in 1988 (from 2,800 in 1983), this will still not be enough to meet the increased demand from industry and academic institutions.

According to NRC, 8.5 per cent of engineering faculty positions are unfilled, and 6,700 new appointments would have to be made to restore student/staff ratios to the levels common in the mid-1970s. The situation is unlikely to improve greatly unless a university career is made more attractive for talented researchers. An associate professor in his 30s, for example, will typically earn less than \$38,000 a year.

NRC calculates that as many as 30 per cent of all US engineers work for the government either directly or indirectly, and uses this statistic to argue that both government and industry would be working for their own benefit in making advanced engineering studies more attractive. Legislation should be introduced that would "facilitate" gifts of laboratory equipment to engineering colleges. And the federal government should be prepared to match dollar for dollar funds raised for new buildings.

NRC makes surprisingly little attempt to

analyse future demands for different engineering skills, but does predict that the number of engineers needed will increase. The lack of hard numbers is blamed on the poor quality of existing databases, and NRC asks in passing that the National Science Foundation (NSF) should do something about it. One somewhat sensitive problem, however, is that more than 40 per cent of graduate students studying engineering in the United States are foreigners on temporary visas, many of whom will return to their own countries after graduating. NRC wants to see more US citizens in the pool.

The NRC study is critical of the way that most federal support for engineering is channelled into a relatively small group of "first tier" colleges specializing in graduate education. More than half of engineering graduates with bachelor of science degrees graduate from the disadvantaged second tier colleges, however.

NRC is laconic when it comes to what exactly should be taught, however. The study concludes, on the basis of verbal arguments, that it might be advantageous to delay specialization in some arcane fields in order to improve management and communications skills, apparently often lacking in engineering graduates; again, NSF is asked to experiment with novel courses.

The NRC study was supported by NSF, the National Aeronautics and Space Administration, the armed forces and industrial corporations.

Tim Beardsley

*Engineering education and practice in the United States: foundations of our techno-economic future. National Academy Press, 1985.

Star wars

Franco-German accord?

THE disagreement at last month's summit meeting between France and West Germany over participation in the US star wars programme seems to be blowing over. President François Mitterrand seems to have offended Chancellor Helmut Kohl at the Bonn meeting by saying that France would not take part, and seems himself to have taken offence at West Germany's willingness, which was taken as a sign of disinterest in the alternative French proposal of a European programme of high-technology research, called Eureka. It is now expected that these differences will melt away when Mitterrand visits Bonn later in the month, but meanwhile it has come to light that two major French companies are planning to go ahead with star wars research in any case. So what exactly did Mitterrand's gesture mean?

M. Pierre Aigrain, research director of Thomson-CSF, one of the two French companies suspected of star wars complicity, said last week that he "didn't know". And others, pointing to the electronics and armaments company's continuing heavy losses, suggested that if Thomson-CSF could (quietly) find a slice of the \$26,000 million star wars budget, nobody in France was going to complain too much.

Aigrain, himself an ex-minister (of research), agrees that, on the whole, Mitterrand is right to be wary of arrangements in which Europe would act merely as sub-contractor to the United States. But would Thomson-CSF itself consider such deals? "That's something we would look at on a case by case basis", said Aigrain. His company is among the world leaders in higher power vacuum-tube technology. "If we were asked to develop a special high power tube, I don't know whether we'd say no. I think probably we'd consider it if the funding's all right and so on. A company will always react by thinking about its own profit." When it comes to research funding from abroad, however, a company would need government approval.

Aigrain considers, as does the French government, that in the Strategic Defense Initiative (SDI or star wars) programme, the United States "is at least as much looking for a way of advancing technology as it is in developing an active weapons system". In Europe, this technology should be developed by European cooperation, Aigrain believes, thus defending President Mitterrand's proposal of a major civil programme, Eureka, to match those aspects of SDI capable of civil application.

"The big problem is that until now, with the exception of the European programme of research in information technology (ESPRIT), Europe has been spending extremely little on joint technology programmes". Even ESPRIT is costing only one-fiftieth of the SDI budget. Thus Mitterrand's Eureka "is a way to say we've no

option but to spend a lot more money on advanced technology. As far as this is concerned I can only say I'm in favour of it", said Aigrain.

The money should not come from extra taxes, but from rechannelling existing, poorly-spent sums. In telecommunications systems, Europe spends more on research and development than the United States and Japan put together, according to Aigrain. But much of the spending is duplicated and "rather ineffective".

The problem with SDI for Europe, says Aigrain, is that the programme has been put under the military. "It's true in every

country in the world that the defence establishment is usually the most efficient user of technological advance. One may not like it but it's true. The problem in Europe is that we don't have a military establishment at European level, but I don't think we should have this until we have a much greater level of political unity."

The most important spin-off from SDI will be in communications and systems, Aigrain believes. "It's obvious — people are always speaking of the basic weapons part of SDI but a much bigger challenge is the automatic collection of information, processing the data, making an intelligent system, says Aigrain. He believes the development and civilianization of such technology may be the biggest effect of SDI.

Robert Walgate

Royal Society

All change ahead in London

THE Royal Society of London seems to be well launched on a period of organizational change, with a new management structure, new people in top posts and with ambitions to exert more influence on public policy. The immediate occasion for the change is the retirement of Dr R.W.J. Keay from the post of executive secretary of the society with effect from 20 May.

Keay is to be succeeded by Dr Peter T. Warren, deputy secretary since 1977. The society has taken this as an opportunity to equip itself with a middle management

society is embarking is that of providing a more systematic commentary on the condition of British science than has been possible by means of the *ad hoc* studies carried out by voluntary committees, which have nevertheless been a growing part of the society's work in the past five years.

The plan now is to set up a small unit, comprising perhaps three professional people, to compile a database describing the pattern of British academic research and eventually to produce analytical papers which, it is hoped, will guide the often acrimonious public debate about the scale and mechanisms of research support in Britain. To ensure the independence of this unit, whose acting head is Dr Peter Collins, the Royal Society plans to meet the costs out of its own funds, not from those supplied by the central government.

Meanwhile, the Royal Society is also preparing for one of its quinquennial changes at the top. Sir Andrew Huxley, president of the Royal Society for the past five years, will retire on 30 November and be succeeded by Sir George Porter, director of the Royal Institution since 1966, who shared a Nobel Prize in 1967 for his work on the use of lasers in the study of rapid chemical reactions. Porter plans to retire from the Royal Institution, but a date has not yet been fixed.

The effects of this change are, as always, unknowable. Sir Andrew Huxley, thought before his election as president to have been shy of public controversy, has been outspoken on a variety of issues, ranging from the Soviet treatment of scientists and the rights and wrongs of cladistics to the British government's regard for basic science. Dogged determination has been the watchword in the past five years.

Porter is more mercurial than Huxley, and has earned a reputation as a popularizer by means of his work with television, which fits in with some of the society's ambitions to improve the public presentation of science. □



Sir George Porter.

structure. The post of deputy secretary has been abolished, but instead there will be three officials responsible for national, international and financial matters.

Mr Peter Cooper, a member of the society's staff for the past ten years, will be the assistant secretary responsible for national affairs, Mr S.J. Cox (now at the British Embassy in Washington) will look after international affairs while Mr N.B. Parfitt, now the society's finance officer, will be in charge of finance and administration. One of the officers of the society says that the need for the new structure has been appreciated for some time, but that the change has been delayed by lack of funds.

Among the new directions in which the

Japanese environment

Rediscovery of pollution

Tokyo

PUBLIC outcry over pollution has almost vanished in Japan as the once notorious Tokyo smog becomes just a memory of the 1970s: the feeling is that the really big problems have been beaten.

The White Paper (policy statement) just released from the Environment Agency thus comes as something of a shock, detailing a host of new and more insidious problems.

There is no doubt that major battles have been won in the control of air pollution. Ten years ago, photochemical smog warnings were issued on close to 300 days of the year and some 5,000 victims a year were being registered in Tokyo alone. In 1965, things got so bad in Yokkaichi, a refinery port close to Nagoya, that all primary school children in the most polluted parts of town were issued with special face masks — not that they appeared to be effective. Now, nitrogen dioxide levels in the air are steadily declining in most parts of the country, despite increased traffic, and smog alerts are down to some 130 days a year.

Where new problems are arising is in the pollution of rivers and lakes and in the mass movement of population to new satellite cities with an infrastructure ill-prepared to receive them. Japan's largest lake, 674 square kilometre Lake Biwa, provides a classic example. The population of Shiga prefecture, in which the lake is located, is growing very rapidly as people move out of the nearby Osaka and Kyoto conurbations in search of a better environment and industries are relocated where land is cheaper.

The resulting effluent pouring into the lake, coupled with acid rain from the huge industrial areas nearby, is now rapidly turning it into a graveyard. The shallow southern half of the lake is in a state of almost total eutrophication, and the northern half is plagued by "red tides" — freshwater algal blooms. Attempts to cut the input of phosphates, by banning sale and use of phosphate detergents, for example, began in 1980 but came too late; it is unlikely that the lake water will show major improvement this century.

Similar problems are seen in many other lakes and rivers, and pollution is spreading into underground waters. Analysis of water in wells shows that some sites are contaminated by carcinogenic organo-chloro compounds at 600 times the levels recognized as safe by the World Health Organization, a serious problem given that 30 per cent of Japan's drinking water comes from underground sources and that the sources of pollution are virtually untraceable with present understanding of underground water flow.

All this highlights the way in which movement of population from the countryside and giant cities into suburban satellite cities, accompanied by an increasing

reliance on automobiles, has not been accompanied by adequate attention to sewerage and water supplies. "A glut of Walkmans and a shortage of sewers" was how experts from the Organisation for Economic Cooperation and Development recently summed up Japan's emphasis on private rather than public expenditure. Noise, traffic jams and garbage disposal problems are also becoming more severe in the new cities as they inherit the crises of the larger cities, and with Japan's urban population expected to reach 70 per cent of the total by the end of the century, there is little hope of a let-up in the pressure. But creating new urban infrastructures, or the greater dispersal of population and industry that the white paper calls for, cannot be achieved without massive expenditure that the government seems unwilling to make.

To cope with deterioration of lake and marshland, the Environment Agency has introduced legal limits on the levels of phosphorus and nitrogen effluent that can be discharged by industry. Japan is the first nation to attempt to control both factors. Initially stringent limits were, however, reduced after opposition by the Ministry of International Trade and Industry (MITI), highlighting the Environment Agency's own lack of power. Although the agency was originally intended as a single comprehensive pollution control body, when it was set up in 1971 it found that ministries with regulatory powers over pollution, particularly MITI, were unwilling to relinquish them. The result is that the agency is a watchdog without teeth: it can plan and co-ordinate policies but is not responsible for enforcing them, except in the case of wildlife protection.

In this area, the Environment Agency has just shown a willingness to respond to international concern by setting up a new "Headquarters for Wildlife Protection Measures". Severe criticism of Japan from abroad, reinforced by the visit of Prince Philip, Duke of Edinburgh, in his capacity as president of the World Wildlife Fund (see *Nature* 311, 698; 1984), for failing to obey the Washington Convention prohibiting the import of endangered species, was the chief stimulus to the establishment of the new office. The number of customs houses through which wildlife can be imported has been reduced to nine and officials are given training in spotting illegal imports.

The white paper itself, however, pays scant attention to international issues. Although it charts the destruction of tropical rain forest in South-East Asia — destruction in which Japanese logging companies and Japan's voracious demand for timber have played a large part — it does not call for any action other than membership of standard international environmental organizations.

Alan Anderson

French universities

New watchdog barks well

Paris

UNDER a dozen glittering candelabra that must each weigh a ton, beneath the frescoed ceilings of the Elysee Palace and surrounded by much of the academic elite of France, President François Mitterrand last Friday gave his full dignity to the latest effort to reform the French university system, the establishment of the Comité National d'Evaluation des Universités, or CNEU.

The purpose was serious, and the guests were hopeful, especially the director-general for the universities at the ministry of education, M. Jean-Jacques Payan, whose cheerful face has been proclaiming lately that he thinks his job now done. The 75 French universities, many created shortly after the 1968 student revolt, and mostly egalitarian and left-wing in spirit, have been opened up to once-hated industry and now, through CNEU, to competition among themselves.

The committee "responds to an evident need" said President Mitterrand on Friday. "It signifies for our country an innovation of major importance." It represents the increasing autonomy of the French universities (sweet words to the relatively powerless university presidents) whose counterpart is "the objective evaluation of their strengths and weaknesses" (rather more bitter words for the feebler institutions).

CNEU will be "entirely free" to make its own judgements and will report each year to the President. M. Mitterrand said, "I expect reports to have concrete effects" on the decisions of the French administration. (CNEU will not directly control and divide funds as does, say, the British University Grants Committee.)

The French universities, described by M. Henri Tézenas du Montcel, who resigned as president of the University of Paris (Dauphine) to write a critical book on his *alma maters*, as those "foggy and smoky" places, are now required, said M. Mitterrand, "to be more transparent and to develop the best among them".

The universities must obey three principles, he said: autonomy, which the President expanded to mean obedience to the recently-introduced system of research contracts agreed between the ministry of education and each university, assessed *a posteriori*; "emulation", a soft word for "competition" in which each university must attempt to emulate the best elsewhere; and quality, "the condition of success in scientific competition... and world technology, which greatly depends on the success of our universities and scientists". On this success the French people depend. And the President depends on CNEU to help increase the quality of the univer-

sities "and therefore the chances for France".

M. Laurent Schwartz, like M. Tézenas du Montcel an old critic of the French universities, is presiding over CNEU, but had raised himself from his sick-bed to deliver his opening address last Friday. He pronounced himself "sensible to the solemnity" that the French President was giving to the installation of his committee, and promised to make of CNEU "an instrument fundamental to the success of the French universities".

According to Schwartz, French universities have been subjected until now to little real objective evaluation, either internally like the US universities, or externally, like the British. In France, there has been only piecemeal evaluation by the research councils, or by the national university promotions committee (the Conseil Scientifique des Universités). CNEU would introduce global evaluation, something "much more ambitious".

"The question is to make known to each establishment, to the minister of education and to public opinion how the universities are doing branch by branch and globally." The better a university does in its evaluation, the more autonomous it should expect to become. CNEU would not distribute money directly, as that might result, in the long term, in "equipartition".

The regulations defining CNEU give it an "entirely new" freedom of movement, said Schwartz. It will have "absolute independence". Many organizations in France are governed by strict rules penned out in minute detail. But "the decree that establishes our committee leaves us on the contrary complete freedom to evaluate as we wish". If only, dreamed Schwartz, this atmosphere of confidence and liberty could extend to other organizations — such as the universities themselves.

"We must combat taboos and corporatism", said Schwartz. "We must understand and make understood: that demands courage and objectivity. We will force ourselves to have them. The grandeur of our task is impressive but is also fascinating."

The 15 members of CNEU will not carry out all the evaluations, but will appoint outside independent experts, said Schwartz. CNEU will use international experts as much as possible "as is done throughout the world". CNEU will not evaluate individuals, but will work at the level of groups and above. It will consider both research and teaching together, "for to separate research and teaching would kill both".

"CNEU will not behave like a policeman" said Schwartz. "Evaluation is totally contrary to Napoleonic control. We shall not be checking that the universities follow all the rules. On the contrary, we shall see if the universities can innovate, if they can launch audacious projects: we shall support initiative and not conformism or retreat."

Robert Walgate

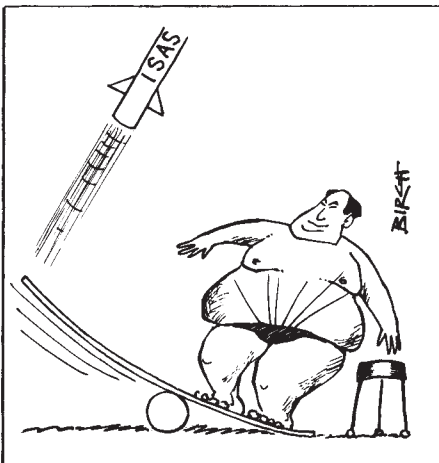
Japan in space

David keeps Goliath guessing

Tokyo

THERE seems no limit to the ambitions of Japan's Institute of Space and Astronautical Science (ISAS), the tiny academic cousin of the government-run giant National Space Development Agency (NASDA). Despite a shoestring budget and home-grown solid-fuel rocket technology, the agency is determined that Japan should be the third country to send a satellite to the Moon.

ISAS has just revealed that it is drafting a detailed programme to launch Japan's first Moon observation satellite in fiscal year 1989. Under the plan, a satellite will be placed in Earth orbit and, after a day for accurate orbital alignment, will be blasted off for the Moon by a "kick motor". On its first pass, the satellite will be slung past the lunar surface at an altitude of about 6,000 km, one month later reaching an apogee of well over 1 million



km. Thereafter, it will be catapulted back towards the Earth-Moon system and, after a second "near miss" of the Moon, will enter a highly eccentric Earth orbit stretching across the Moon's path. Two months and four Earth orbits later, the satellite will then whip off on another two-month loop around the Moon, or so the theory goes, but according to ISAS's "gunnery lieutenant" Hiroki Matsuo, professor of orbital dynamics, much more detailed calculations will be required before he can predict exactly where Japan's first Moon shot will end up.

Given ISAS's resources, it is a wonder that this organization can even contemplate a lunar mission. Japan is unique in having two space organizations, each equipped with its own satellites, rockets and launch sites. The larger NASDA, affiliated to the Science and Technology Agency, is in charge of the development of applications satellites and their launch vehicles, while ISAS, affiliated to the Ministry of Education, Culture and Science and operating on a fraction of NASDA's budget, carries out research and development of scientific satellites and launch vehicles. NASDA uses

liquid fuel rocket technology developed almost entirely under US licence but ISAS has its own "home-made" solid fuel launchers.

In the 1960s, when ISAS was part of Tokyo University rather than a national institute, the Japanese government asked the institute to limit the size of its rockets, to avoid competition with NASDA and to prevent the academic organization getting too big for its boots or so it said. By the early 1970s, ISAS had already reached the 1.41-m limit on the tail diameter of its rockets, since when its MU-series rockets have been getting taller and taller but no wider, at least at the base. With the Moon shot, however, ISAS is reaching the limits of ingenuity in what it can do with its present launchers.

To kick the lunar probe out of Earth orbit and off to the Moon, ISAS plans to add a 500-kg fourth stage to the MU-3SII used in the successful launching of the Halley's comet test satellite, *Sakigake* (see *Nature* 17 January, p.175). Add to this the 170-180 kg weight of the satellite and the payload capabilities of MU-3SII will be stretched to the full, whence ISAS's cunning plan makes gravity do most of the work. Launch dates and total budgets for the programme have not yet been agreed but ¥160 million (\$0.6 million) has been approved for systems design in the current fiscal year.

Unlike previous programmes, the lunar programme, codenamed MUSES (MU Space Engineering Satellite), is aimed largely at engineering studies of control of the speed and orbit of the satellite and, according to Professor Matsuo, no scientific instruments will be carried on the first satellite, MUSES-A. However, a possible successor, MUSES-B, may carry a scientific package to be dropped to the lunar surface.

But the big question in the minds of the institute's members is what more can be done with present launch facilities. Among the projects under consideration are a solar physics project for observations of solar flares that would, ideally, be carried out at the next solar maximum in 1991, but which would clash with a plan, in conjunction with the US National Aeronautics and Space Administration, to launch a satellite from the space shuttle which would follow a similar profile to MUSES-A. So the solar physics project will probably be scheduled for 1992.

Also under consideration is a project to examine diffuse X rays following on the successful series of X-ray satellites, while the institute also has more grandiose ambitions to send satellites to the planets which would be beyond the capabilities of MU-3SII. Using NASDA launchers would help, but NASDA has its hands full while ISAS cherishes its independence.

David Swinbanks

French research

End to growth in sight?

MORE is not enough for the French national research council, CNRS (Centre National de la Recherche Scientifique). Despite a real increase in its spending since 1981 of over 35 per cent, a figure only to be dreamed of on the other side of the English Channel, CNRS is pressing the ministry of research for a further increase this year. And that increase should be double last year's, at least in support for laboratory equipment and materials, and approach a real 10-12 per cent annually for the next three years, CNRS director-general Pierre Papon is claiming.

But research minister Hubert Curien, whose new three-year plan for the future of French science is now before the prime minister Laurent Fabius, has warned CNRS that it may have to be content with what it has. And with the French government facing a possible trade deficit this year as opposed to a planned surplus, and Fabius refusing to win votes in next year's parliamentary elections with a spending spree, Curien may well be right.

Papon, however, says this may mean cuts in the CNRS 5-7 year plan announced recently (see *Nature* 2 May, p.5), in which 20 research areas were selected for special effort. That plan itself required some hard choices, claims Papon, and to develop it requires the re-equipment of some laboratories, particularly in mini-computing and other "middleweight" expenditure.

But has the period of plenty at CNRS, which amounted to an average real increase of 5.5 per cent a year in total budget for the five years 1981-85, not left the organization sitting pretty, with cash to spare? Not according to some outside observers, who judge that President Mitterrand's policy of "solving the economic

crisis" by spending on science and technology has left basic science, the fields mostly supported by CNRS, protected from cuts, but not truly expanded. The 5.5 per cent "real" increase against French domestic inflation has been swallowed up by a falling French franc against harder currencies in which most scientific equipment and materials must be bought (typically dollars, marks and Swiss francs).

Nevertheless, CNRS is attempting to share equipment costs, and technicians, by creating "federated institutes" around well-instrumented laboratories, linking three to five existing groups around genuine joint research objectives. This will have the double advantage of reducing costs and of unifying an often fragmented French research effort. But still "money is the big problem" Papon says, thus shedding a disturbing

light on those research councils elsewhere whose budgets have remained fixed if not falling.

Innovations other than more money that CNRS would like to see in the new plan, which should go before parliament in July, include an increase in the number of studentships for people studying for one of the new PhD degrees, more incentives to mobility of scientists, and the establishment of more CNRS-linked laboratories in the *grandes écoles*, the elite engineering schools which tap the best talent emerging from French secondary education.

Although these schools produce some 12,000 engineers a year, only 5-7 per cent go on to do a PhD (or the previous equivalent, "third cycle" diplomas), according to CNRS figures. This lack of scientific training is a "real handicap" for French industry (of which the graduates of the *grandes écoles* often become leaders), according to Papon.

Robert Walgate

Charitable foundations

Wellcome Trust plans asset sale

THE British medical charity the Wellcome Trust, whose only substantial asset consists of 100 per cent of the shares in the Wellcome Foundation, the successful pharmaceutical manufacturer, is planning to increase its income and diversify the source from which money flows by disposing of a fifth of its holding in the company.

The immediate attraction of the proposed share sale is that the trust could expect to realize a substantial sum of money from the sale which, invested in high-yielding securities, might even double its income, which is estimated to amount to £23 million during the present year. During 1984, the Wellcome Foundation earned a profit (after tax) of £48.6 million on a turnover of £806 million.

The share prices of comparable companies, such as Glaxo, are at present not very different from 20 times the net earnings per share, which would value the Wellcome Foundation at something like £1,000 million.

A statement put out by the trust earlier this week emphasizes, however, that diversification is also a large part of the trustees' motives in disposing of part of their shareholding. The trust says that the trustees have "for some time been concerned about the wisdom of having all their eggs in one basket". But the statement also says that after the disposal of 20 per cent of the shares, the trust will not sell further shares for at least two years, and that it will not allow its shareholding to fall below 50 per cent.

The chances are that the new arrangement will also benefit the foundation, whose attempts to behave as a thoroughly commercial organization have always been cramped by the relationship with the trust. The relationship has, among other things,

prevented the company from rewarding its senior employees by means of share options and from growing more quickly by the acquisition of other companies in return for its own stock. The second constraint will remain so long as the trust retains a controlling interest.

The relationship between the trust (which is technically a charitable foundation) and the foundation (which is a commercial company) derives from the will of Sir Henry Wellcome, who died in 1936 leaving to the trust which he created the sole ownership of the company, originally Burroughs Wellcome Ltd. The company was renamed the Wellcome Foundation in 1926.

Over the years the relationship between the trust and the foundation has been curiously ambivalent. Each has been jealous of its independence from the other, with the company repeatedly in difficulties in explaining to its dependent charity why its annual share of the commercial profit has to be substantially less than 100 per cent. (In 1984, the trust received £17 million of the total profit of £48 million, most of this by way of payments under deeds of covenant allowing the company to economize in tax payments.)

Even so, the trust is now the largest of the private foundations in the United Kingdom.

In a quite separate development, Sir John Vane, research director at the Wellcome Foundation for the past eleven years, announced last week that he is to leave the company so as to devote himself to his scientific interests. Vane, who was awarded the Nobel prize for his account of the mechanism of action of aspirin, said last week that he has no immediate plan to do something else. □

CNRS spending

Over the period 1981-95 inclusive, the spending of the French CNRS (which supports 10,000 of its own scientists, 25,000 engineers and technicians, and many more scientists by means of grants at universities) is estimated to have increased (in real terms) at average annual rates of:

- 2.9 per cent in nuclear and particle physics (excluding international subscriptions).
- 5.4 per cent in other basic physics and mathematics.
- 8.4 per cent in physics for engineering.
- 5.1 per cent in chemistry.
- 4.1 per cent in Earth sciences and astronomy.
- 6.0 per cent in life sciences.
- 6.0 per cent in social science and humanities.
- 14.5 per cent in interdisciplinary programmes. □

UK agricultural research

Penury brings radical reappraisal

ADVERSITY (mostly financial) has persuaded the most threatened of the British research councils, the Agricultural and Food Research Council (AFRC), to canvass a radical plan of reorganization. One proposal would reduce the number of separate institutes from about twenty (with seven separate Scottish research institutes) to perhaps half that number.

It is also proposed that there should be a management board for agricultural research to which would belong research council officials and a representative of the Department of Agriculture and Fisheries, Scotland, as well as the "ten or fewer" institute directors.

The discussion document, *A long-term view*, says that the number of research institutes would be reduced "by successive amalgamation and restructuring", and that wherever possible institutes will be sited on or near a university campus. Under such a regime, institute directors would have a greater degree of independence in the direction of management of the research for which they were responsible. The document suggests that it should be possible, within such a framework, to give research groups more flexibility, by specifying "the questions to be answered rather than the areas of science to be studied".

Part of the council's management problem is that many of the research institutes for which it is the chief source of financial support are not formally directly under its control, but are historically independently constituted. The Rothamsted Experimental Station is one of these. Thus, in the discussion document, AFRC suggests that people whose salaries it pays should in future be its own employees, which would entail both a change of status and, for some people, a greater degree of insecurity. No doubt the council would benefit through the greater freedom it would have to move people from one place to another.

The discussion document says little about its immediate origin, the proposed reduction of funds for research commissioned by the Ministry of Agriculture, Fisheries and Food of £10 million in the current financial year and twice as much during 1986-87. The question is to be considered by the priorities board for research in agriculture at a meeting later in the summer, but it appears that there is very little prospect of relief from the Treasury edict that these substantial sums of money should be lopped from what has become, in effect, part of the council's budget.

Among the other proposals put forward in the discussion document is the suggestion that the need to avoid duplication of research effort within Europe may make it necessary for the council to withdraw from some fields of research.

But the council is plainly also hoping that it would win research support from

elsewhere for some of the research that it carries out at present. The document also repeats the council's expressed intention, during the past several years, of increasing its income from research contracts with private industry as well as from other government departments than those responsible for agriculture.

On people, the council promises to consult trades unions on the reorganization outlined in the document, and says that it acknowledges the need that creative scientists should have a secure environment in

Tumour necrosis factor

Drug agency jumps TNF gun

Washington

In an unusual move, the US Food and Drug Administration (FDA) has allowed human tumour necrosis factor (TNF) produced by recombinant DNA technology to be given to a cancer patient. FDA had previously maintained that there are insufficient animal data on the effects of TNF to approve clinical trials, but it has now allowed Dr Herbert Oettgen of Memorial Sloan-Kettering Cancer Center in New York to treat a gravely ill patient with TNF manufactured by the Asahi Chemical Industry company of Japan.

In the normal course of events, FDA grants a so-called Investigational New Drug exemption — permission to enter clinical trials of a putative therapy — only when there are sufficient data to provide a reasonable expectation that the therapy will be safe and effective. FDA allowed a compassionate exemption for the Sloan-Kettering patient on condition that Sloan-Kettering's institutional review board accepts full responsibility for the case, since FDA had no prior information on the TNF used, and the data submitted with the compassionate exemption request covered only basic safety requirements such as pyrogenicity and sterility.

FDA itself appears to be confused about the exemption. Even after granting Sloan-Kettering permission to use TNF on its patient, FDA officials were insisting that the substance was not being used on patients in the United States.

The Sloan-Kettering patient is said to be a member of the staff of the institution. The request to FDA for a compassionate exemption was approved unanimously by over 30 members of Sloan-Kettering's institutional review board on 9 April; according to Dr John Lewis, chairman of the board, FDA was provided with a treatment protocol, a detailed clinical assessment of the patient's condition and "voluminous" information about TNF.

Dr Doris Hutchison, head of the institute's investigational drug committee, said that FDA's bureau of biologics had

which to work. But it also says that it needs to plan for change, for which reason it will continue to recruit many of its staff on short-term contracts.

Flying a more adventurous kite, the council says that part of its case for continued existence is that AFRC is responsible for the "largest non-medical biological research capability" in Britain, which will be widely taken as a reference to the fondness of Sir Ralph Riley, the recently-retired secretary of the council, for the creation of a new research council with responsibility for basic biological research, medical and non-medical.

Comments on the discussion paper are invited by 15 July. □

been "very co-operative" in dealing with the request.

FDA not infrequently grants Investigational New Drug exemptions on compassionate grounds when the relevant animal data have been submitted for FDA's consideration but are held up in the bureaucratic pipeline. Patients with acquired immune deficiency syndrome (AIDS) have been treated under compassionate exemptions with drugs approved for other conditions, for example. Compassionate exemptions have also been granted for trials on seriously ill patients who fall just outside the age range specified in the protocol for a clinical trial. It is, however, less common for FDA to approve what amounts to a clinical experiment when the data are as fragmentary as they are for recombinant human TNF. Some authorities on medical ethics speculate that FDA might feel justified in making an exception if the patient concerned has a sophisticated understanding of the risks and benefits of the treatment.

Dr Lloyd Olds, associate director of scientific development at Sloan-Kettering, is one of the original researchers on TNF, first produced from mouse serum. Dr Olds is being supplied with genetically engineered human TNF for research purposes by Genentech Inc.

Although this material is being used only for animal and *in vitro* studies, according to Genentech, Asahi's TNF is undergoing clinical trials in Japan. After the publication of scientific reports detailing the cloning and expression of the human TNF gene (*Nature* 312, 721 and 724; 1984 and 313, 803; 1985), researchers at Genentech and Asahi were deluged with enquiries from anxious cancer sufferers and their families about where TNF could be obtained.

It is still unclear how soon organized clinical trials of TNF will start in the United States, or whether the Sloan Kettering case will serve as a precedent for future compassionate exemptions in similar cases.

Tim Beardsley

Halley's comet in ancient times

SIR — In his comment on the Babylonian observations of Halley's comet¹, C.B.F. Walker states that in Mesopotamia "almost all the early texts are concerned with astrology rather than astronomy"². Walker continues by noting that the earliest astronomical observations known to Ptolemy in *circa* AD 150 dated "from the time of the Babylonian king Nabonassar (747–734 BC)". Both these points deserve comment.

First, although doubts have been expressed as to the link between astrology and astronomy in Babylonian times (for example, ref. 3), there is no firm evidence that the powerful temple priests of Mesopotamia made any distinction between what we call by the separate names of "astrology" and "astronomy": facility in expounding the former necessitated skill in understanding the latter.

Second, Sawyer and Stephenson showed in 1970 (a hypothesis confirmed by Muller and Stephenson in 1975)⁴ that what was in all likelihood a total solar eclipse was observed during daytime at Ugarit on 3 May 1375 BC. It seems fair to assume (although the details were not known to Ptolemy in AD 150 and are still not known fully to us now) that astronomical observations took place in and around Mesopotamia for a considerable period of time before 747 BC.

MARTIN PULBROOK

Department of Ancient Classics,
St Patrick's College,
Maynooth, Co. Kildare, Ireland

1. Stephenson, F.R., Yau, K.K.C. & Hunger, H. *Nature* 314, 587 (1985).

2. *Nature* 314, 576 (1985).

3. Clagett, M. *Greek Science in Antiquity*, 11 (Books for Libraries Press, Plainview, New York, 1971).

4. Stephenson, F.R. and Clark, D.H. *Applications of Early Astronomical Records* (Adam Hilger, Bristol, 1978), 43–44.

Embryo research

SIR — Professor J.A. Davis (*Nature* 25 April, p.666) wonders whether a "legally or morally valid distinction" can be made between negatively interfering to stop a natural process, for example by procuring the abortion of a fetus *in utero*, and simply refraining from "positive intervention required for its survival", by not putting an egg fertilized *in vitro* into a woman, without which it must inevitably die.

But there is at any rate a *legal* difference between intentionally killing someone and doing nothing to save him. You are under no legal obligation to rescue a drowning man, and if you feel morally obliged to jump in to help him that is a matter for your own conscience, and no concern of the law. But to push him in deliberately is certainly a criminal offence, and probably murder if he drowns.

Professor Davis rightly understands that the argument in my last letter (*Nature* 14

March, p.126) could not be used to justify aborting an embryo that had already started on its normal development *in utero*, but that it might allow experimentation with embryos generated *in vitro* which, undoubtedly human as they are, cannot of themselves realize their potential for further development without some positive act of intervention from outside.

The Warnock Committee (*Report*, para. 11.22) proposed a limit of 14 days for keeping an embryo alive *in vitro* (otherwise than frozen), whether or not it was used for experimental work, and nobody seems to have suggested that this should be much extended. But it should be noted that experiments with living fetuses that have been legally aborted before the age of viability, that is to say up to something over 20 weeks gestation age, are not prohibited under English law. These are by definition doomed to die but they can be kept alive for at least several hours, and considerable publicity was given some years ago to attempts at Cambridge to develop an artificial placenta, using a human fetus which from press photographs appeared to be of 16–18 weeks gestation age. Although the Warnock Report (p.64) has suggested that the law ought to be reconsidered in this respect, such work is at present still quite legal, even if it is not for the squeamish.

C.B. GOODHART

University Department of Zoology,
Downing Street,
Cambridge CB2 3EJ, UK

Artificial hearts

SIR — Your leading article "Hearts in the wrong place" (*Nature* 21 March, p.206) classifies you among the modern legal luddites, glorifying form over substance and accepting the idea that the lawyer-regulators know best, regardless of the consequences of their actions.

You admit that Dr Copeland's patient would clearly have died without the use of the artificial heart. In the light of this, neither the patient nor the patient's family was harmed by the heart's insertion and important information was obtained which may well allow the effective use of such hearts or improved models in the future.

There was also the possibility, albeit remote, that the artificial heart might have preserved the patient's life for a long enough time to allow for a successful implantation of a new human heart.

Thus the only potential results of the use of the heart were beneficial, even your editorial writer being unable to discover anyone who had been harmed. The use of available technology in such situations should be encouraged and not barred. In view of the admitted benefits and lack of harm to anyone, Dr Copeland deserves commendation not condemnation.

School of Law, JAMES B. BOSKEY
Seton Hall University,
1111 Raymond Boulevard,
Newark, New Jersey 07102, USA
©1985 Nature Publishing Group

Value-free science

SIR — Mark Diesendorf (*Nature* 10 January, p.92) questions the notion of value-free science, which I discuss in my letter of 27 September 1984 (p.294). Unfortunately, his comments suffer from the very confusion of categories that I tried to clarify. Since this error has marred much of the discussion of the subject, and since my explanation was excessively brief, it seems worthwhile to consider further what we mean by "science" and by "value-free" or "objective".

The word "science" is ordinarily used to refer to three things: a methodology, a resulting body of knowledge and the ensemble of activities of those who professionally use that methodology. The methodology has evolved a set of canons, and a social framework, designed to ensure maximal objectivity and reliability of the resulting knowledge. On the other hand, the activities of scientists are indeed filled with subjective value judgments: by the individual (what problem to work on, how to approach it, how to present the results), and also by social organizations (what to support, what to accept for publication, how to translate scientific knowledge into technological practice). But despite this subjective element, if the science is honest the practitioner must try to make its *content* — the data and the logical inferences drawn from them — as objective as is humanly possible. He may not succeed perfectly, but at least he should not deliberately inject ideological or other biases.

Diesendorf further calls attention to such presumably value-laden terms as "fashionable" and "confusion" in my letter. I would suggest that these terms are actually capable of being objectively assessed. But more fundamentally, value-laden terms would not be out of place. For discussions of the nature of science constitute still another category, to be distinguished from the three noted above: they are metascience, presenting philosophical judgments rather than objective knowledge.

Finally, I agree with Diesendorf that problems involving social value judgments should not be treated as though they were purely technological, and his example of planning how to generate electricity fits. But I believe he errs in similarly categorizing the risk of fallout from nuclear weapons. It is a technical question, whose answer then becomes part of the broader question of what to do about these weapons. With the growth of technological innovation we face an increasing number of such questions (for example, the dangers of biotechnology), and we impair our analysis if we do not try to separate their technical from their social aspects.

BERNARD D. DAVIS

Bacterial Physiology Unit,
Harvard Medical School,
Boston, Massachusetts 02115, USA

New ways with microscopes

The range of the phenomena usable in the construction of microscopes continues to increase. Neutrons, for example, are now being used in place of photons.

IF you have a particle or a field of some kind whose interaction with material objects varies with their constitution, the chances are that you will be able to fashion a microscope from the phenomenon. That truth is nicely illustrated by the versatility of microscopy based on the electromagnetic fields corresponding to optically visible radiation. Contrast between different elements of an object can depend on reflectance, on absorption during transmittance through a thin section, on rotation of the plane of polarization or on variations of refractive index, as in phase-contrast microscopy. The texture of an image is determined by the contrast, but a useful microscope also requires a means of magnification equivalent to the lenses of the familiar microscope. Two new developments this week suggest that people's ingenuity is far from exhausted.

The report by a group of Spanish molecular biologists and Switzerland-based IBM researchers on p. 253 of this issue is an application to a biological problem of a technique of microscopy previously used for the measurement of the surface structure of semiconductor materials and others used in the construction of electronic devices, and which may itself be the only application to microscopy of a strictly quantum phenomenon. Electrons, the textbooks say, will tunnel through a potential barrier if there is a state of identical energy for them then to attain. What is called the scanning tunnelling microscope is for practical purposes a device for arranging that the tip of a fine electrode will physically follow the profile of a surface at a distance of 10 ångströms or less.

In the usual application to the measurement of the profiles of semiconductor surfaces, the voltage between the electrode and the semiconductor is maintained at a value sufficient to promote an electron from the filled valence band to one of the energy states of the largely unfilled conduction band (usually a mere fraction of a volt), but which is less than the work function of the material of which the electrode tip is made, and which then serves as the potential barrier. The electrode tip can then be made to follow the surface profile by means of circuitry, including a piezo-electric crystal, designed to keep the tunnelling current constant, and at a value which may be no more than a few nano-amperes. For all the world, the device is the electronic analogue of the engineering devices for measuring successive profiles of a surface by ampli-

fying electro-mechanically or otherwise the displacements perpendicular to the surface of a small ballbearing that is rolled along in contact with it.

The sensitivity of the quantum tunnelling effect is vividly illustrated by the way in which Baró *et al.* show a series of profiles along a surface of pyrolytic graphite which indicate a step-like structure corresponding to the abrupt termination of a couple of layers of the carbon atoms accounting for less than 7 ångströms of thickness.

Indeed, the resolution of the microscope is reckoned to be of the order of 1 ångström perpendicular to the surface and between 5 and 10 ångströms laterally. Baró *et al.* explain clearly enough how they have been able to correlate the known shapes (from electron microscopy) of bits and pieces of the bacteriophage $\phi 29$ dried on a graphite surface with the measured tunnel profiles, but their promised account of the mechanism of conduction through these biological molecules could be of great interest in its own right. Their technical achievement, however, is to have made the microscope work in air at ordinary pressure, which should make this type of instrument more versatile even in the measurement of semiconductor surfaces.

The other new development is the first realistic account of a neutron microscope, a goal long advocated by the Soviet physicist I.M. Frank but now realized by a group at the Technical University of Munich and the Laue-Langevin Institute at Grenoble (Herrmann, P. *et al. Phys. Rev. Lett.* **54**, 1969; 1985). This microscope uses "ultra-cold" neutrons from the source at Grenoble, while the microscope itself consists of a pair of mirrors — a large parabolic mirror 20 cm in diameter and a smaller (5 cm) mirror which is convex and spherical.

Ultra-cold neutrons are neutrons moving very slowly, some 6 metres a second in this case. Their interest for would-be neutron microscopists is that they can be reflected from suitable surfaces as if they were photons, which is a simple consequence of the circumstance that, at speeds like this, their wavelength is greater than the internuclear spacing in materials such as nickel-58 (used for coating the "optical" elements in the Munich design).

The snag is that the trajectories of neutrons moving so slowly will be affected by gravity, which is why the two mirrors are arranged in a vertical direction, with the

secondary mirror above the primary (and each of them fixed in a horizontal plane with a precision of 10^{-4} radians). Even so, neutrons moving vertically will be decelerated, so that neutrons starting out with slightly different speeds will, left to themselves, come to a focus at different points, which is the equivalent in this field of what those who design optical instruments know as chromatic aberration.

It turns out that the chromatic aberrations of the two mirrors will cancel out only if the neutrons reflected from the parabolic mirror are allowed to reach the peak of their trajectory, at a height of 2 metres or so, and then allowed to fall back onto the secondary mirror, whose reflecting convex surface must therefore point upwards, like the larger primary.

The system has been designed to yield a magnification of 50, with the object placed near the optical focus of the parabolic mirror. The image is formed after reflection from the secondary mirror some 1.5 metres above the primary, whence neutrons are reflected sideways (and downwards, so that they can pick up a little speed) into a neutron detector, which simply counts the numbers arriving. Herrmann *et al.* say that a suitably faceted mirror deflecting neutrons from different parts of the field into different detectors would allow almost instantaneous observations.

So far, they have only the most humdrum observation to report, that of the profile of the neutron intensity across a slit placed in the object plane. The measured resolution of this neutron microscope is 0.1 mm, but that, the authors say, is determined by the counting errors with the low-intensity source of ultra-cold neutrons now available. (Grenoble plans 100 times as much intensity soon.) Aberration with the present arrangement should limit resolution to about 0.02 mm, but preselection of the velocity of the neutrons should allow that to be reduced.

But to what end? The authors' dream is that the reasons and techniques that have made the scattering of thermal neutrons a powerful means of telling the distribution of hydrogen in matter of all kinds can be translated simply into neutron microscopy. But one guesses that a large part of their motivation must be a little like the familiar reason for climbing Everest. Nobody else has made a neutron microscope function at a reasonable magnification, although Frank's Soviet group is well on the way.

John Maddox

Quasi-crystals

Respectable icosahedral symmetry

from Paul A. Heiney

THE structure and properties of icosahedral 'quasi-crystals' were among the topics discussed at the recent meeting of the American Physical Society (Baltimore, 25-29 March, 1985). Quasi-crystals seem to represent a new state of matter in which icosahedral point symmetry is combined with a modified form of translational order. The clue to their structure may lie in the properties of Penrose tilings of a plane (see ref. 1).

Icosahedral ordering was first observed (D. Shechtman, National Bureau of Standards) in rapidly quenched alloys of Al-Fe, Al-Mn, and Al-Cr (ref. 2). Dendritic microcrystals up to 2 μm in size show the sharp electron diffraction patterns more usually generated by crystals, but with icosahedral rotational symmetry (see figure). Elementary geometry shows that icosahedral symmetry is forbidden for crystalline solids. The usual explanation of electron diffraction peaks which are crystallographically disallowed involves multiple scattering from twinned microcrystallites, but in the present case, the twinning hypothesis appears to be denied by both convergent-beam electron microscopy and dark-field imaging, which are consistent with local icosahedral symmetry on length scales as short as 200 Å.

Much of the more recent work has centred on optimizing conditions for the growth of large-grain pure samples in the Al-Mn system (R.J. Shaeffer, National Bureau of Standards and L. Bendersky, Johns Hopkins University). The icosahedral phase is produced either by quenching a stream of molten alloy on a rapidly spinning copper wheel or by sweeping an intense electron beam across the surface of the alloy. If the cooling rate is too great, there are fewer icosahedral grains and they are smaller; if it is too low, ordinary crystals of various Al-Mn compounds are formed. The stability of the icosahedral phase is enhanced by a small amount of Si. An unexpected new 'T' phase, formed at higher Mn concentrations, may be intermediate between the icosahedral phase and a normal crystal.

High-resolution X-ray powder diffraction measurements (P. Bancel, University of Pennsylvania) provide data for quantitative tests of various theories of icosahedral order. The X-ray diffraction peaks are described by an icosahedral indexing scheme with six Miller indices. It is inferred that the positional correlation lengths of several hundred angstroms, extracted from peak widths, are reduced by defects which nevertheless allow orientation order to be maintained across grains with dimensions of 1 μm . High-resolution transmission electron microscopy (R.

Gronsky, Lawrence Berkeley Laboratory and L. Bursill, Arizona State University) shows details of the atomic structure. Images formed from the electron diffraction patterns by optical Fourier transformation are remarkably similar to those produced by the quasi-crystal models now developed; 5-fold atomic packing symmetry and defects such as dislocations and stacking faults are observed.

In the best-known theoretical model³ for these materials, two or more unit cells are packed with unequal spacings to form a 'quasi-crystal' without normal crystalline translational symmetry (P.J. Steinhardt, University of Pennsylvania). Quasi-crystals are much more highly ordered than glasses, but have special symmetries that are impossible for crystals. Remarkably, although quasi-crystals are not periodic, a calculation of the diffraction pattern predicts sharp spots, as with a crystal. In an alternative approach⁴ to icosahedral order,

quasi-crystalline lattices can be generated by projections from higher dimensions (D. Gratias, CECM/CNRS); the icosahedral lattice is generated by projecting a six-dimensional hypercubic lattice onto three dimensions. This mathematically elegant approach allows a straightforward calculation of X-ray diffraction intensities.

In another approach⁵⁻⁷, based on Landau's theory of the emergence of symmetry during phase transitions such as solidification, the atomic structure is described as a set of mass density waves with adjustable magnitudes and phases. An icosahedral quasi-crystal is generated by six density waves directed toward the vertices of an icosahedron. This method lends itself naturally to calculations of the structural stability, thermodynamics, elasticity and the occurrence of defects in the icosahedral phase. A careful treatment of dislocations (D. Levine, University of Pennsylvania) reveals that these defects are considerably more complicated than their crystalline analogues; each dislocation requires two vectors, rather than one, to describe it fully.

Conventional discussions of matter in the solid state which assume either crystallinity or almost total disorder are not easily applicable to quasi-crystals. Future research will address fundamental questions about the nature of vibrations, electron transport, mechanical strength, thermodynamic equilibrium and other properties of these new materials. The immediate challenge for experimentalists is to produce larger, more pure and better-characterized samples; and for theorists, is to develop the mathematical tools needed to deal with this intriguing new state of matter. □

1. Maddox, J. *Nature News and Views* 313, 263 (1985).
2. Shechtman, D., Blech, I., Gratias, D. & Cahn, J.W. *Phys. Rev. Lett.* 53, 1951 (1984).
3. Levine, D. & Steinhardt, P.J. *Phys. Rev. Lett.* 53, 2477 (1984).
4. Elser, V. (Bell Laboratories preprint).
5. Bak, P. *Phys. Rev. Lett.* 54, 1517 (1985).
6. Levine, D. *et al.* *Phys. Rev. Lett.* 54, 1520 (1985).
7. Mermin, N.D. & Tröter, S.M. *Phys. Rev. Lett.* 54, 1524 (1985).

Paul A. Heiney is in the Department of Physics and Laboratory for Research on the Structure of Matter, University of Pennsylvania, Philadelphia 19104, USA.

IMAGE
UNAVAILABLE
FOR
COPYRIGHT
REASONS

High-resolution transmission electron micrograph of 5-fold plane of quenched 6:1 Al-Mn. Bar, 2nm (courtesy R. Gronsky and L. Tanner).

Cell motility

Fast axonal transport dissected

from Dennis Bray

ANYONE interested in cell motility likes to see things move. Electron micrographs have their place, as do two-dimensional gels. But for innocent enjoyment nothing beats a working model. Myofibrils isolated from a muscle contract explosively when ATP is added; bundles of microtubules from a sperm flagellum propagate waves and swim around. Marvellous! And now the machinery driving yet another type of cell movement — that of particle transport in the cytoplasm — has been isolated. As shown on page 245 of this issue¹, and in

several other recently published papers, all you really need is a membrane vesicle and a microtubule.

For some time, it has been suspected that microtubules are involved in cytoplasmic transport. Membrane-bound organelles moving through a cell (see figure) are usually close to a microtubule, and drugs that disrupt microtubules often, but not always, paralyse these movements. Whole-cell studies of this kind have now been taken to their logical extreme by the discovery of an organism in which movement of organelles

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

Cultured chick embryo neurites show fast transport of organelles, some of which are visible as dense particles with phase-contrast optics. These two micrographs show a typical neurite at an interval of 10 seconds; the arrows denote an organelle which has moved along the neurite. Scale bar, 15 μm . (Courtesy of P. Hollenbeck.)

is confined to a single microtubule².

The organism was discovered on the sides and bottom of a freshwater aquarium in Berkeley, California. Fortunately, whoever found it must have been something of a naturalist, as they welcomed the intruder with a microscope rather than a scrubbing brush. The organism was composed of an enormously branched network of fine threads in which myriads of tiny, phase-dense particles streamed. Following a natural line of enquiry, the threads — subsequently identified as the giant freshwater amoeba *Reticulomyxa* — were examined in the electron microscope and found to resemble nothing so much as the thin processes of nerve cells. Like axons, each cytoplasmic thread contains aligned microtubules, and some are so thin that they contain but a single microtubule.

Correlating their light- and electron-microscope observations, Koonce and Schliwa were able to show that the phase-dense organelles, most of which are mitochondria, can move even in threads that contain only one microtubule². Moreover, they can move in either direction, despite the fact that the microtubule is certain to have a distinct structural polarity. The authors conclude that explanations of organelle movement based on the beating of myosin crossbridges in muscle, or of the dynein side-arms in a cilium, both of which produce unidirectional forces, are not to be accommodated wholesale.

But any cell, no matter how attenuated, is still something of a black box. Three years ago, a far more accessible system was described that allows the rapid transport of vesicles to continue in the absence of an outer cell membrane³. Axoplasm is extruded from the giant axon of a squid and then examined by video-processed polarization-based light microscopy. With this exciting and powerful method, it is possible to see swarms of tiny vesicles, 0.2 μm or less in diameter, scurrying about their business

in the axoplasm. If the axoplasm is spread even more thinly the vesicles can be seen moving along individual fine filaments, the nature of which forms the subject of two papers recently published in *Cell*^{4,5}.

Organelles ranging in size from mitochondria down to the smallest vesicles all move along 'transport filaments' in the extruded axoplasm at steady rates of about 2 $\mu\text{m s}^{-1}$. Unlike the movements in intact axons, large organelles travel as quickly as small ones, and the movements are smoothly continuous, lacking the saltatory, stop-and-go motion long considered the hall-mark of cytoplasmic particle transport. The difference, presumably, is that in the intact axon many organelles are obstructed by other components of the cytoskeleton. Most importantly, the transport filaments, although no more than 25 nm in diameter, are clearly revealed by the high-technology light microscope to be microtubules. Treatment and examination of the filaments by immunofluorescence and by electron microscopy shows that they contain tubulin (and not actin) and that they have the unmistakable substructure of microtubules.

Given these observations, what experiment would you like to see performed next? If your answer is to test whether movement is regained when separated vesicles and microtubules are reconstituted, turn to the paper by Gilbert *et al.* on page 245. Rather than try to separate microtubules from axoplasm, they used the ready-made parallel bundles of microtubules in the flagella of sea-urchin sperm. Freed of their outer membrane by detergent and dialysed against low-ionic strength solutions to remove most of their dynein side-arms, these readily available bundles present smooth straight tracks of uniform (and known) polarity. Gilbert *et al.* show that vesicles recovered from the squid axon travel smoothly, rapidly, with-

out interruption and (as in both the giant amoeba and isolated axoplasm) in either direction along these tracks. Because movements proceed in the absence of most of the dynein arms, and because they ignore the intrinsic polarity of the ciliary axoneme, it is unlikely that the vesicles are moving by the same machinery that drives the cilium. More and more it seems that each organelle must have its own power source, like an onboard motor, that can drive it in either direction along a microtubule. The motor does not resemble that driving muscle contraction or ciliary beating; so what might it be?

Close on the heels of all these studies comes yet another instalment in the unfolding story. Vale and colleagues⁶ have now developed a reconstituted transport system in which the components are split not just two ways, but three. Membrane organelles from squid axoplasm constitute one part, and isolated microtubules the second — in this case purified from squid optic lobe tubulin. But the third component is the most exciting: a soluble, heat-labile, trypsin-sensitive extract without which nothing moves. Addition of this extract enables not only organelles but also polystyrene beads to travel along microtubules. It even causes the microtubules themselves to glide over the surface of a glass coverslip, looking like miniature snakes. Any speculation as to the nature of the mechanochemical protein in the soluble extract is clearly redundant; the answers are surely already in the press. □

1. Gilbert S., Allen, R.D. & Sloboda, R.D. *Nature* **315**, 245 (1985).
2. Koonce, M.P. & Schliwa, M. *J. Cell Biol.* **100**, 322 (1985).
3. Brady, S.T., Lasek, R.J. & Allen, R.D. *Science* **218**, 1129 (1982).
4. Vale, R.D., Schnapp, B.J., Reese, T.S. & Sheetz, M.P. *Cell* **40**, 449 (1985).
5. Schnapp, B.J., Vale, R.D., Sheetz, M.P. & Reese, T.S. *Cell* **40**, 455 (1985).
6. Vale, R.D., Schnapp, B.J., Reese, T.S. & Sheetz, M.P. *Cell* **40**, 559 (1985).

Dennis Bray is on sabbatical at the University of Otago Medical School, Dunedin, New Zealand.

Thunderstorms

Wind shear and aviation safety

from Edwin Kessler

THAT aircraft should avoid thunderstorms is advice as old as flying itself. Although airborne weather radar provides a means of inflight evaluation of the weather, flight through echo-weak areas of thunderstorm complexes has led to several fatal and highly publicized accidents. All but three of the twelve such accidents between 1940 and 1970 followed a catastrophe at substantial altitude. With subsequent emphasis on the use of radar for avoiding storms, the incidence of accidents initiated at altitude declined remarkably. Since 1970, US aircraft have been involved in seven more fatal thunderstorm-related accidents, but five of these occurred during takeoff and landing and have been attributed to strong wind shears, mostly associated with thunderstorms near airports. There have also been other disturbing incidents during aircraft

operations near terminals. The focus of research has accordingly shifted to the study of both thunderstorm downdrafts and outflows (downbursts) and of the strong variations of horizontal wind that accompany intense warm fronts¹.

Whereas aircraft used to bounce around a lot in bad weather, like leaves in a storm, today's planes are more sensitive to larger-scale atmospheric phenomena. The typical 1950s propeller-driven transport had a relatively low wing loading: it flew about 300 m.p.h. at altitude and about 100 m.p.h. on landing. The reciprocating engines responded within about 2 seconds to pilot inputs. Today's commercial transports fly at about 600 m.p.h. at altitude and 150 m.p.h. at landing and takeoff, and their engines take up to 7 seconds to respond fully to pilot inputs. Thus, during takeoffs

and landings, the 1950s aircraft would have flown about 300 feet before the engines responded; today's aircraft fly about 1,500 feet. And today's runways, about 10,000 feet long, twice the length required for a typical piston-engine aircraft, indicate the extended exposure of modern commercial aircraft to boundary-layer phenomena during takeoff and landing.

The variation of wind divided by the distance over which the variation occurs is the wind shear: mathematically, the shear is a tensor whose nine components correspond to the three orthogonal directions in which the wind variations can occur, combined with the three directions from which the wind can be blowing. The pilot is most interested in wind and its variations in the directions of flight, and in the vertical wind, because these produce the principal lift forces. Small-scale shears, termed turbulence, can produce discomfort, but if an aircraft enters a region of much reduced headwind during takeoff or during approach to landing, loss of lift is a serious safety concern. This is exacerbated when a downdraft is encountered along with a sudden unfavourable change of horizontal wind speed. In a severe downdraft outflow, with wind variation around 20 m.p.h per 1,000 feet, decline of headwind and downdraft can cause irreversible loss of altitude to the point of a crash. This is most likely during takeoff or landing when a thunderstorm is over or near the airfield, but can

also occur with solitary atmospheric waves.

As a result of several incidents beginning in the late 1960s, the Federal Aviation Administration (FAA) issued a series of advisory circulars on the hazards of thunderstorms and wind shear, and developed new instrumentation for use at airports. Until the late 1970s, surface winds at air terminals were reported by a single centrally located anemometer. Guidance from a single device is inadequate in the jet age, especially when winds vary by 50 m.p.h or more from one side of an airport to the other, and such variations can even occur in clear air and without local visual clues. In 1976, the FAA developed the Low Level Wind-shear Alert System — a system of multiple anemometers with a display and alarm system that notifies the control tower operator when any one of the anemometers indicates a wind speed different from the centre field wind². The efficacy of the system is suggested by the fact that only one fatal accident (in July 1982 at New Orleans) has been attributed to wind shear in an airfield with the equipment. About 60 airfields now have the basic system, and there are plans for installation at 50 more. The installations at Denver and New Orleans are being upgraded by adding extra, more closely spaced anemometers.

Substantial study continues to refine our knowledge of low-level wind-shear events, their individual and statistical characteristics, climatology and predictability. Downbursts and microbursts are being studied in the Chicago area^{3,4}, at laboratories of the National Center for Atmospheric Research and the National Oceanic and Atmospheric Administration (NOAA) at Denver, Colorado⁵, and at NOAA's National Severe Storms Laboratory at Norman, Oklahoma^{6,7}. Downdraft outflow phenomena are unusually frequent and intense at ground level in the Denver area. There they are often abetted by a layer of rather dry air on and near the ground, having a steep lapse rate of temperature. Such a boundary layer tends to amplify any thunderstorm or shower downdraft, especially with the strong evaporative cooling that accompanies precipitation from scattered showers. Findings from the Denver-based projects JAWS (Joint Airport Weather Studies) and CLAWS (Classify, Locate and Avoid Wind Shear) have both theoretical and practical implications. Downdrafts strong enough to be classified as microbursts around Denver are often 0.5–1 mile in diameter, and typically last 5–15 minutes, although occasionally they develop significantly within one minute. The difference between peak winds on opposite sides of outflow on the ground at the time of maximum development is about 55 m.p.h. In summer, there is on average one microburst a day within 14 miles of Denver airport.

Further work seeks to develop cost-effective systems, both for observing hazardous microbursts in appropriate detail and for communicating the information to pilots at the time of landing and taking off, so that

accidents will be avoided and economies are achieved in the use of time and fuel. Observed atmospheric extremes are also being programmed in flight simulators to evaluate aircraft control strategies for mitigating effects of extreme shear during unforeseen encounters.

Other atmospheric phenomena hazardous to flights at low altitudes include solitary waves, strong vertical shear of the horizontal wind and vortices rotating around vertical axes along gust fronts. Solitary waves can retain significant intensity while propagating for hundreds of miles in a stable boundary layer; when the air is dry they may be practically invisible, but under suitably moist conditions they are marked by a line of cloud that may have a deceptively innocuous appearance. They have been especially targeted for research in north-eastern Australia, where they are known as 'morning glories' and are quite common before noon⁸. In the United States they sometimes develop from thunderstorm gust fronts that propagate in a stable air layer ahead of the originating disturbance⁹.

Intense warm frontal shear — a variation of the horizontal wind with height — especially when accompanied by poor visibility in rain and fog, can cause a descending aircraft to land short of the runway — as happened on 17 December 1973 at Logan Airport, Boston¹⁰. This outcome of extreme conditions can usually be averted by timely advice to the pilot on wind speed and direction at different altitudes. Doppler radars can provide this and other detailed wind information routinely, at intervals of a few minutes^{11,12}.

Future weather-surveillance systems for airports will probably include surface anemometers and Doppler radar, and possibly a lightning-locating system. It will be essential to collect data at a central location and to process them there for communication to pilots. As technology advances, we can expect an improved match between timely displays of processed meteorological data, actual wind and weather, aircraft capabilities, and pilot performance^{13–15}. □

1. Lee, J.J. in *The Thunderstorm in Human Affairs* (Univ. Oklahoma Press, 1983).
2. Goff, R.C. Rep. No. FAA-RD 80-45; FAA-NA-80-1, National Aviation Facilities Experimental Center (US Dept of Transportation, Atlantic City, 1980).
3. Fujita, T.T. *J. Atmos. Sci.* **38**, 1511 (1981).
4. Wakimoto, R.M. *Mon. Weath. Rev.* **110**, 1060 (1982).
5. Wilson, J.W., Roberts, R.D., Kessinger, C. & McCarthy, J. *J. Clim. appl. Met.* **23**, 898 (1984).
6. Goff, R.C. *J. Aircraft* **14**, 423 (1977).
7. Rust, W.D. & Doviak, R.J. *Nature* **297**, 461 (1982).
8. Christie, D.R. & Muirhead, K.J. *Aust. Meteor. Mag.* **31**, 97 (1983).
9. Doviak, R.J. & Ge, R. *J. Atmos. Sci.* **41**, 2560 (1984).
10. National Transportation Safety Board, Rep. No. NTSB-AAR-74-14 (1974).
11. Doviak, R.J., Rabin, R.M. & Koscielny, A.J. *IEEE Trans. Geosci. Rem. Sensing* **GE-21**, 25 (1983).
12. Hogg et al. *J. Clim. appl. Meteor.* **22**, 807 (1983).
13. *Low Altitude Wind Shear and Its Hazard to Aviation* (National Academy, 1983).
14. Suomi, V.E., Fox, R., Limaye, S.S. & Smith, W.L. *J. Clim. appl. Meteor.* **22**, 766 (1983).
15. Reynolds, D.W. *Bull. Am. meteor. Soc.* **64**, 264 (1983).

Edwin Kessler is at the National Severe Storms Laboratory, National Oceanic and Atmospheric Administration, Norman, Oklahoma 73069, USA.



100 years ago

Paradise Found. The Cradle of the Human Race at the North Pole. A Study of the Prehistoric World. By William F. Warren, S.T.D., LL.D., (London: Sampson Low and Co.)

It has come to be an understood thing that when geologists or biologists propound theories as to past stages of life on the earth, and these theories attain to a certain popularity, some theologian shall twist the words of the Book of Genesis into a new interpretation, to show that this was what the inspired author meant all the time. A fresh musician has set Moses to dance to a new scientific tune. Since the publication of well-known modern views as to the diffusion of plants and animals from the Polar Region, it was to be expected that we should have a book proving that man was created in an Arctic Paradise with the Tree of Life as the North Pole; and here the book is. Other ancient cosmologies, such as the Greek and Indian, are made to bear their not always willing testimony. The commendatory letters published from Professors Sayce, Tiele, and Whitney do not at all imply that these eminent scholars countenance the Polar Paradise doctrine. The author seems to have sent them a paper on "Ancient Cosmology and Mythical Geography," their acknowledgements of which they are now perhaps hardly delighted to find as certificates in a "Paradise Found."

From *Nature* 32 28, 14 May 1885.

Neurophysiology

Potential challenge from glia

from A.R. Gardner-Medwin

THE neurones in our brains are required, as we all know, for the complex information-processing tasks of everyday life. Roughly an equal amount of space in the brain, however, is devoted to glial cells, chiefly astrocytes and oligodendrocytes, whose functions are diverse and poorly understood. Glial cells offer many challenges in understanding the overall function of nervous tissue, just one of which is their intriguing electrophysiological behaviour. Several recent papers¹⁻⁵, including one on page 229 of this issue by Bevan and Raff¹, show that glial cells in culture sometimes have hitherto unsuspected membrane characteristics, including high permeability to potassium, higher membrane conductance than resting neurones, direct electrical connections with one another, and the ability to respond metabolically to depolarization induced by local neuronal activity, all of which add to their fascination. How far these properties are characteristic of glial cells *in situ* is still a matter for conjecture.

The electrophysiological study of glial cells in tissue culture began in 1958 with work of Hild, Ching and Taskai⁶ who showed that morphologically identified glial cells do not display the action potentials that are characteristic of most neurones, yet have resting potentials that are sensitive to potassium. Over the years, with contributions from many people working on both isolated cells and intact tissues, a picture has emerged in which some glial cells have very remarkable properties⁷⁻¹⁰, including probably the most extreme selectivity of membrane permeability for potassium over sodium of all known biological cells.

One aspect that is beginning to be well characterized is the role of glial cells in stabilizing extracellular potassium levels in the nervous system¹¹⁻¹⁵. Many questions remain, even in this highly specific area. For example, the relative importance of potassium dispersal through glia and of net uptake into glial cells remains controversial. The principal mechanisms of net uptake are uncertain and the significance of all these mechanisms for normal neural function are largely subjects for speculation. These current issues would tax one's ingenuity enough in designing experiments to settle them, even without the addition of the surprising new data from studies on

isolated glial cells in culture.

The special advantages of work in tissue culture are that the cells can be studied in a simplified context and with the widest range of modern cell biology techniques. These include antigenic characterization with monoclonal antibodies and 'patch' electrophysiology, in which a pipette is sealed tightly to the outside of the cell membrane. Because glial membrane conductance is largely a potassium conductance in most preparations, it is gratifying that K⁺-selective single channels (with, interestingly, a variety of different sizes) have been identified in oligodendrocytes². The observed channels open and shut intermittently, although any channels that are constantly open may perhaps have escaped detection for technical reasons.

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

A type 2 astrocyte in culture, stained with fluorescently labelled antibody to glial fibrillary acid protein (courtesy of Dr. J. Cohen).

First of the particularly novel glial characteristics to emerge are sodium-selective³ and calcium-selective⁴ conductances, revealed with various pharmacological interventions. Astrocytes treated with barium and tetraethylammonium ions can even produce action potentials, involving Ca²⁺ channels⁴ and with some resemblance to action potentials of heart muscle. Voltage-dependent sodium⁵ and potassium¹ conductances, comparable with those normally found in neurones, have now been demonstrated in apparently normal, well-characterized astrocytes in culture (see figure). Despite many electrophysiological studies of cultured astrocytes⁷, such potentials have not previously been reported, so there would seem to be substantial variation in properties between glial cells in different laboratories, and even to a certain extent among cells prepared in a single laboratory, with consistent techniques⁵. The reasons for these differences are obscure, but an impor-

tant consideration may be that the cells for which results are illustrated in Bevan and Raff's study¹ seem to have much higher input resistances (100-150 MΩ) than have been found before (usually 1-10 MΩ). It is not clear whether this could be explained by morphological differences, variations in the extent of coupling between cells or aspects of the recording conditions that may affect the membrane conductance. The new findings¹⁻⁵ do show convincingly, however, that some glial cells are capable of producing the molecules, presumably proteins, responsible for various different channels found in membranes.

At least three suggestions can be made as to the possible functional significance of voltage-dependent channels in cultured glial cell membranes. They might have no functional significance at all, appearing only as a consequence of particular culture conditions; they might exist in mature glial cells *in vivo* and play some physiological role; or they might exist only transiently in glial cells and play some role in the development or maintenance of neurones⁵. Because the channels are opened only when the membrane is depolarized above about -40 mV, they could function solely in conditions leading to extreme glial-cell depolarization.

Such extreme disturbances are known to occur only in two pathological conditions: spreading depression, a condition believed to occur in some migraine attacks¹⁶, and anoxia. The potassium conductance of glial cells may play a significant role in preventing spreading depression^{17,18}, which would indeed be assisted by a conductance increase on depolarization, but none of the studies *in vivo* has yet revealed a class of glial cells with non-linear conductance⁷⁻¹⁰. The recent studies on cultured glial cells may spur attempts to tackle the substantial technical problems that will be encountered before such cells can be identified with certainty *in vivo* and distinguished from neurones. □

1. Bevan S. & Raff. M. *Nature* **315**, 229 (1985).
2. Kettenman, H., Orkand, R.K. & Lux, H.D. *Pflügers Arch.* **400**, 215 (1984).
3. Bowman, C.L., Kimelberg, H.K., Frangakis, M.V., Berwald-Netter, Y. & Edwards, C. *J. Neurosci.* **4**, 1527 (1984).
4. MacVicar, B.A. *Science* **226**, 1345 (1984).
5. Bevan, S., Chiu, S.Y., Gray, P.T.A. & Ritchie, J.M. *J. Physiol., Lond.* **361**, 85P (1985); *Proc. R. Soc. B.* (in the press).
6. Hild, W., Chang, J.J. & Taskai, I. *Experientia* **14**, 220 (1958).
7. Kuffler, S.W., Nicholls, J.G. & Martin A.R. *From Neuron to Brain* (Sinauer, Sunderland, Ma., 1984).
8. Sears, T.A. (ed.) *Neuronal-Glial Cell Interrelationships*, Life Sci. Res. Rep. **20** (Springer, Berlin, 1982).
9. Treherne, J.E. (ed.) *J. exp. Biol.* **95**, (1981).
10. Walz, W. & Hertz, L. *Prog. Neurobiol.* **20**, 133 (1983).
11. Coles, J.A. & Orkand, R.K. *J. Physiol., Lond.* **340**, 157 (1983).
12. Dretzel, I., Heinemann, U., Hofmeier, G. & Lux, H.D. *Exp Brain Res.* **46**, 73 (1982).
13. Gardner-Medwin, A.R. *J. Physiol., Lond.* **335**, 393 (1983).
14. Newman, E.A. *Nature* **309**, 155 (1984).
15. Walz, W., Wuttke, W. & Hertz, L. *Brain Res.* **292**, 367 (1984).
16. *Current Views on Spreading Depression*. *Anais Acad. brasil. de Ciencias* **56**, 371 (1984).
17. Hertz, L. *Nature* **206**, 1091 (1965).
18. Gardner-Medwin, A.R. *J. exp. Med.* **95**, 111 (1981).

Erratum

In the article by Jonathon Bacon 'Why flying locusts do not crash' (*Nature* 9 May, p. 94), the fourth paragraph in column 3 should begin "The descending neurones make only weak direct connections with flight motor neurones but have strong inputs..."

A.R. Gardner-Medwin is in the Department of Physiology, University College London, Gower Street, London WC1E 6BT, UK.

Atmospheric physics

Predicting the ionosphere

from D.G. Cole

THE status of the ionosphere — modelling, measurement techniques, applications and forecasting of the ionized upper atmosphere — was discussed recently at a conference held under the auspices of the Australian Ionospheric Prediction Service in Sydney*. Regarded by some as an anachronism, ionospheric research has enjoyed a resurgence of interest to cope with a demand for high-speed digital data traffic on ionospherically propagated paths, increased high frequency (3–30 MHz) radio communications in developing countries and trans-ionospheric propagation problems.

The fundamental tool of the ionospheric forecaster is an ionospheric model. The present status of such modelling was reviewed by J. R. Dudeney (British Antarctic Survey). At present the most widely accepted model is the International Reference Ionosphere (IRI) (eds Lincoln, J. V. and Conkright, R. O. NOAA Report, Boulder, 1981) which is distributed on magnetic tape. IRI gives profiles of electron and ion density, temperature and relative ion composition; a user supplies location, time of day, season and solar activity. The model is used widely, even though it is inapplicable to auroral zone regions and times of very high solar activity, and does not consider irregularity structures.

The two regions that still provide the greatest problems in any modelling or prediction schemata are the equatorial and polar/auroral zones. Of these, the equatorial region is the easier to model. Indian researchers have gathered much decisive data by widespread low-cost satellite-beacon scintillation and electron-content studies. The IRI model has been shown to underestimate considerably the low-latitude F-region — the ionospheric layer of maximum electron density at about 300 km above ground level — daytime plasma density scale-height and total electron content. Disagreement with the topside ionosphere is bad, which is relevant to transionospheric systems and, through vertical plasma transport, to HF ionospheric systems.

Total electron-content predictions can be improved by using theoretically calculated profiles, but this is prohibitively time-consuming and expensive even with present-day large computers. D. N. Anderson (US Air Force Geophysics Laboratory) and coworkers have recently made considerable progress with a semi-empirical low-latitude ionospheric model that uses only coefficients from such theoretical profiles to form the basis of a fast portable computer code to produce realistic low-latitude F-region electron-density profiles.

The polar region and the behaviour of irregularities still remain a world-wide problem, requiring concerted intensive global data collection and analysis. An exercise which can be regarded as a forerunner of such a study, project SUNDIAL, was conducted late in 1984. The project arose because there was no prediction capability for day-to-day variations in ionospheric structure and it highlights the fact that the various ionospheric domains are not isolated subsystems, but are indeed interactive elements in the entire solar-terrestrial network. The project looks to progressive development of a genuinely predictive ionospheric specification capability under both quiet and disturbed conditions. Such a capability would add direct solar and magnetosphere inputs as well as latitudinal and longitudinal coupling to the fundamental ionospheric model, and requires near real-time updates from a responsive network of ionospheric monitoring stations.

The experimental period of project SUNDIAL was based on an eight-day flight of the space shuttle carrying a 1.28-GHz ground imaging radar in October 1984. This aperture-synthesis radar requires phase-path constancy through the ionosphere to obtain maximum resolution. During this period the international ground-based ionospheric programme (coordinated by E. P. Szuszczewicz, Science Applications International, Virginia) collected ionogram, electron content and

scintillation data from equatorial, mid-latitude and both polar domains, later supplemented with data on solar, magnetospheric, geomagnetic and interplanetary activity. Two workshops are planned in 1985 to analyse these data and to issue guidelines, recommendations and suggest follow-on programmes. Future studies will emphasize new programmes and also the appropriate coordination to ensure that diverse existing ionosphere measurement facilities are fully used. Interestingly, the USSR Solar Terrestrial Physics group was the first to propose an intense global ionospheric study.

A final note of interest is the detection of meteor echoes by the Australian Project Jindalee, an Over-the-Horizon Backscatter Radar near Alice Springs. Conventional radar meteor studies, starting with the early work at Jodrell Bank in 1945, have always used only the direct line-of-sight return signal. It seems now that, for the first time, significant rates of meteor echoes have been detected by various ionosphere (and ionosphere-ground) reflection modes. R. M. Thomas (Project Jindalee team) also suggests that the steerable, narrow beam-width antenna pattern, coupled with the frequency, agility and sophisticated processing available to the system, can be used to optimize the radar for a particular meteor radiant by changing the appropriate parameters. Such a facility could significantly revitalize interest in delineating the faint-field meteor influx and improve the management of polar high-speed meteor forward-scatter links of interest to military and other circles. □

D.G. Cole is in the Ionospheric Prediction Service, 162-166 Goulburn Street, PO Box 702, Darlinghurst, New South Wales 2010, Australia.

Evolution

The point of a toucan's bill

from J.S. Jones

MODERN evolutionists are beguiled by random change. Recent theories about DNA sequence divergence suggest that natural selection does not determine the fate of most genes and that genetic drift is the main agent of evolution, with selection acting only to remove harmful mutations. It is therefore important to remember that not all evolution happens by accident; that natural selection is a potent force for adaptive change, and that its products can be seen all around us. Some of the most spectacular examples of the intensity of natural selection and its ability to produce rapid adaptation have emerged from recent studies of birds ranging from the mundane sparrow to the outrageous toucan.

House sparrows were introduced into the United States in 1852, and are now widespread. Since their introduction, American sparrow populations have undergone considerable morphological evolution, and

northern birds are larger and have relatively shorter extremities than do those in the south¹. These differences are maintained in the face of dispersal sufficient to produce relative uniformity in the frequencies of genes coding for soluble enzymes². They probably represent an adaptive response to climatic selection: birds with short limbs survive in cold weather considerably better than do those with long. In one particularly harsh Kansas winter a 25 per cent difference in mean fitness was associated with humerus length³; a difference related to the extent of heat loss through the extremities. Larger males, but small females, are better at surviving the winter — an effect which might result from an advantage of more dominant males and more submissive females in conditions where fighting is metabolically costly. Differences in food items selected by birds of different body size may also help to ex-

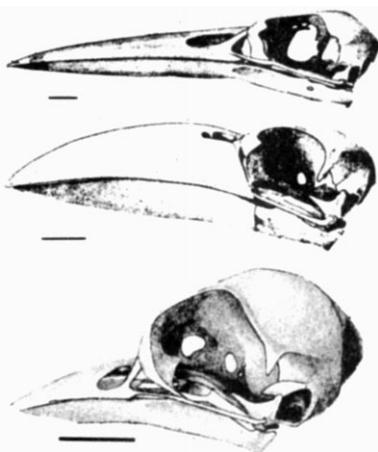
*The conference on the ionosphere and radio wave propagation was held at the University of Sydney, 11–15 February 1985, sponsored by the International Union for Radio Science.

plain the greater sexual dimorphism found in northern populations⁴.

Natural selection appears to be an effective agent in producing rapid adaptation in these introduced populations. There is no information on the genetic basis of morphological variation in sparrows, but transfer experiments with other American birds suggest that some geographical changes in body form are a direct response to the environment⁵. Consequently, estimates of heritability are needed before the evolutionary significance of such changes in morphology can be assessed. As recently discussed by Paul Harvey⁶, information of this kind is now available from new work on Darwin's Galapagos finches⁷.

Patterns of differentiation in their soluble enzymes suggest that the finches have occupied the islands for less than a million years and have diverged rather rapidly from the emberizine finches that are their mainland ancestors⁸. There are striking morphological differences between species, from the long slender bill of *Certhidia olivacea* to the stout and powerful beak of *Geospiza magnirostris*. Assuming there is no strong correlation between genetic and environmental variation in wild birds, family studies suggest that the heritability of morphological characters, such as body size or beak dimensions, is high^{7,9}. For example, three quarters of the overall variation in *G. fortis* body size is due to genetic variation. There is therefore abundant genetic variation on which natural selection might act. Periodic droughts, such as those on Isla Daphne Major in 1977, 1980 and 1982, can lead to mortality rates of 85 per cent. Much of this is selective, and adult survivors have substantially larger bodies and beaks than those who die, probably because they can feed better on the hard seeds that persist through the drought. A selective shift of six per cent in morphological traits can occur in one year¹⁰.

Selection of this intensity, acting on highly heritable characters, has the potential to generate rapid morphological change. A new study of the numbers of finch species on the various islands and their patterns of differentiation in beak shape in relation to food availability shows that coexisting species always differ in bill dimensions by at least 15 per cent. Such differences can be explained only by interspecific competition and by local adaptive radiation into a variety of feeding niches¹¹. The role of competition for food in driving adaptive radiation is particularly manifest when comparing the morphology of species present in sympatry and in allopatry on different islands. The bills of *G. fortis* and *G. fuliginosa* diverge from each other to a much greater extent in mixed populations than in single-species populations. Comparing of feeding behaviour with availability show that this character displacement results from exclusion from particular food items in mixed populations, leading to a change in optimal beak size for each species¹². Natural selection on beak



Adaptations in skulls of Coraciiform and Piciform birds. Top to bottom: *Alcedo atthis* (kingfisher); *Selenidera langsdorffi* (toucan); *Sasia abnormis* (piculet). Scale bars, kingfisher and piculet, 10mm; toucan, 5mm (from ref. 17).

form appears to be intense enough to bridge the gaps between existing species within only a few years. This evidence that selection is the directing force in evolution on the Galapagos contrasts with the emphasis placed on accidental change in evolutionary studies of Hawaiian *Drosophila*¹³.

Adaptive radiation has the potential to obscure patterns of taxonomic affinity if unrelated organisms, living in ecologically similar circumstances, evolve body forms sufficiently alike to produce an appearance of common ancestry¹⁴. This is true to some extent among Galapagos finches¹⁵, but is far more striking among Australian birds. The Australian passerines include many species with names (wrens, crows, babblers and honeyeaters) reflecting their similarities in structure and behaviour to birds in other parts of the world. Until recently, this diversity was thought to reflect a series of invasions of Australia by a number of unrelated ancestors. Now, however, the use of DNA hybridization to measure genetic affinity suggests that it results from an explosion of adaptive radiation from one endemic ancestor within the past 60 Myr¹⁶, to fill the niches occupied by wrens, warblers, flycatchers and chats in other parts of the world. Unique forms such as bowerbirds, butcherbirds and bellbirds have also evolved. Once again, natural selection in diverse habitats has led to a rapid adaptive response.

Comparisons of structure and of ecology have recently been combined to illustrate the forces of natural selection that have led to an exuberance of form in a group of birds far more diverse than sparrows, finches or Australian passerines¹⁷ (see figure). The Coraciiformes and Piciformes include 15 families. Some are familiar — kingfishers, bee-eaters, woodpeckers and toucans; others are less well known — todies, cuckoo-rollers, jacamars, barbets, puffbirds and piculets. Despite their name, most kingfishers do not fish. Instead they sally for terrestrial prey, which may include small snakes. The beak is large and stout

— which is perhaps not surprising given that some species nest in tunnels excavated by flying directly at a river bank. Bee-eaters pursue insects on the wing, and can catch up to 350 in a single day. Their bill is less heavily constructed than the kingfisher's but is not as lightly built as that of the hoopoe, which probes the soil for insects. The huge beak of the hornbill is used to crush dangerous prey and to peel fruit; food items, once prepared, are tossed into the air and caught in the bird's open mouth. Honeyguides have a short but robust bill and can feed on wax. The huge casque of the toucan is used to peel fruit, but may also be useful in intimidating other birds when robbing their nests, and as a social signal. Finally, the wrynecks, piculets and woodpeckers feed on insects obtained by probing or excavating with a straight chisel-like bill. This diversity of skull form is accompanied by an equivalent radiation of the muscles of the head and neck, which are reduced in bee-eaters, but massively strengthened in woodpeckers. Other specializations include the extremely small tongue of kingfishers and the brush-like tongue of the toucan, which is among the largest in any bird. This rare combination of information on structure, ecology and behaviour in a widely distributed and highly variable group gives an unusual insight into the power and opportunism of selection in leading to adaptive change.

In 1825, Sydney Smith wrote of the toucan: "To what purpose is a bird placed in the forest of South Cayenne, with a bill a yard long, making a noise like a puppy dog, and laying eggs in hollow trees? The toucans, to be sure, might retort — to what purpose were gentlemen in Bond Street created? . . . There is no end to such questions. So we will not enter into the metaphysics of the toucan." Smith was hasty in his dismissal of this enigmatic bird: modern ornithology reminds us that molecular biology is still little more than an account of the structure of the genome, and that we should be wary of abandoning natural selection as an agent of change until we can describe the functional anatomy of molecules in as much detail as is now possible for beaks, muscles and feathers. □

1. Johnston, R.F. & Selander, R.K. *Evolution* 25, 1 (1971).
2. Fleischer, R.C. *Evolution* 37, 1001 (1983).
3. Fleischer, R.C. & Johnston, R.K. *Nature* 298, 747 (1982).
4. Fleischer R.C. & Johnston, R.K. *Can. J. Zool.* 62, 405 (1984).
5. James, F.C. *Science* 221, 184 (1983).
6. Harvey, P. *Nature News and Views* 314, 13 (198x).
7. Grant, P.R. *Biol. J. Linn. Soc. Lond.* 21, 113 (1984).
8. Patton, J.L. *Biol. J. Linn. Soc. Lond.* 21, 97 (1984).
9. Grant, P.R. *Proc. R. Soc. B* 220, 219 (1983).
10. Price, T.D. *et al. Nature* 309, 787 (1984).
11. Schluter, D. & Grant, P.R. *Am. Nat.* 123, 175 (1984).
12. Schluter, D. *et al. Nature* 227, 1056 (1985).
13. Carson, H. & Templeton, A.R. *A. Rev. Ecol. Syst.* 15, 97 (1984).
14. Blondel, J. *et al. Evol. Biol.* 18, 141 (1984).
15. Schluter, D. *Evolution* 38, 921 (1984).
16. Sibley, G.C. & Ahlquist, J.E. *Curr. Ornithol.* 1, 245 (1983).
17. Burton, P.J.K. *Bull. Brit. Mus. (Nat. Hist.) Zool.* 47, 331 (1984).

J.S. Jones is in the Department of Genetics and Biometry, University College London, London NW1 2HE, UK.

Plant geology

Changing weight of photosynthesis in the ocean

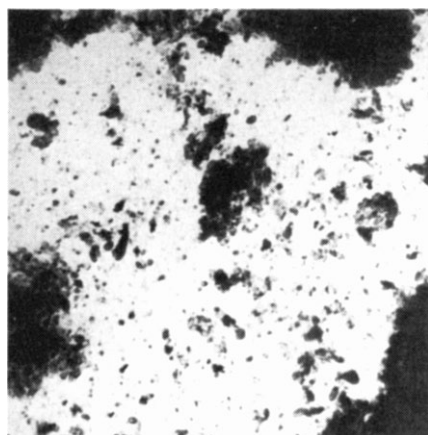
from I. Gilmour

ON PAGE 216 of this issue Arthur *et al.*¹ provide further evidence for the debate on what controls the carbon isotope composition of organic matter in marine sediments. The common photosynthetic pathways discriminate against the heavy carbon isotope (^{13}C), mainly as a result of a kinetic isotope effect inherent in carbon fixing reactions. As carbon dioxide dissolved in seawater contains more ^{13}C than atmospheric carbon dioxide, marine organic carbon is generally isotopically heavier — that is, ^{13}C enriched — than most land plants or terrestrial carbon. This has led to a general belief that the variations in the carbon isotope composition of organic matter in marine sediments provide a measure of the relative amounts of marine and terrestrially derived organic matter^{2,3}. Arthur *et al.*, however, now report an apparent reversal of this general trend in some marine sediments of Cretaceous and older ages¹ (see figure). Using a second, independent, geochemical parameter to evaluate the source of the organic matter, they report carbon isotope values for rocks apparently containing mainly marine organic matter that are lighter (that is, more ^{13}C -depleted) than those containing mainly terrestrial organic matter.

The parameter that Arthur *et al.* have measured is the hydrogen content of the organic matter, determined using the Rock-Eval pyrolysis method, which provides a 'hydrogen index' related to the H/C ratio in the organic matter⁴. Terrestrial organic matter has a lower H/C ratio compared with marine organic matter because of the presence of lignin. This material, which gives land plants their 'woody' characteristic but is not found in marine algae, has a low H/C ratio, reflecting its more aromatic chemical structure. Arthur *et al.*, by plotting the carbon isotope composition of the organic matter against the 'hydrogen index' or H/C ratio, relate organic carbon isotope values to the chemical composition of the organic matter, which in turn is dependent on the relative contributions of marine or terrestrial material. Using this approach, they are able to demonstrate that for Cretaceous and older organic rich sequences a negative correlation is commonly found, suggesting that the lightest carbon isotope values are related to chemical compositions that indicate the greatest contributions of marine organic matter. For the more recent Cenozoic sediments, they observe either a positive correlation or no correlation, reflecting the 'normal' isotope variations between marine and terrestrial materials and purely marine organic matter, respectively. Thus, pre-Cenozoic

marine organic matter is anomalously depleted in ^{13}C by around 5 to 7 parts per thousand compared with marine organic matter in more recent sediments.

Anomalous ^{13}C depletions in ancient sediments are fairly common, particularly in Precambrian sediments⁵⁻⁷ where different carbon cycling pathways, notably those based on methane, have been invoked to explain them. It is difficult to try to explain such anomalies in ocean sediments, where the organic matter is derived predominantly from phytoplankton that use carbon dioxide. Arthur *et al.* consider several possibilities for this anomaly. They discount diagenetic effects, which generally do



Organic material from a Cretaceous black-shale sequence from the Falklands Plateau, South Atlantic, which has revealed lighter carbon isotope composition than expected. The material is predominantly the remains of photosynthetic marine algae with minor amounts of material derived from the continents of the time. (Photo courtesy of I. Gilmour.)

not produce sufficiently large shifts in the carbon isotope composition of the organic matter. They also eliminate, although not totally, the possibility that changes in the carbon isotope composition of the organic matter reflect changes in the dominance of marine phytoplankton groups because this explanation is not supported by the Cenozoic palaeontological record. The three remaining possibilities all relate to one factor, the level and isotope composition of dissolved carbon dioxide in the oceans and its possible effect on photosynthesis. The authors eliminate a secular variation in carbon isotopes because the average Cretaceous carbonate is about 1.5 parts per thousand heavier than modern pelagic carbonates, which would result in marine organic matter being heavier, not lighter. Thus, the favoured interpretation is as follows: increasing the carbon dioxide concentration in the oceans can have a pro-

found effect on photosynthesis. Photosynthetic carbon isotope fractionation is maximized under conditions of high carbon dioxide availability^{8,9} and, as Arthur *et al.* point out, this would result in ^{13}C -depletion of organic matter. An increase in carbon dioxide availability can be accomplished by reducing the ocean temperature, which increases the solubility of carbon dioxide. But the Cretaceous oceans were as warm, if not warmer, than present-day oceans. Thus, Arthur *et al.* argue that possible higher levels of atmospheric carbon dioxide during the Cretaceous, combined with lower levels of surface productivity, would have resulted in more available carbon dioxide and increased fractionation of the carbon isotopes found in marine plankton.

Are these interpretations correct? If so, then modern marine photosynthesis may be occurring under geologically unusual conditions. True or false, their approach is certainly on the right lines. By relating carbon isotope composition to H/C ratio and hence to chemical structure, the relative contributions of marine and terrestrially derived material are better understood. However, the carbon isotope values obtained still represent an average of two components, whereas ideally the marine and terrestrial material should be resolved completely. Failing this, the approach adopted by Arthur *et al.* is certainly an improvement on merely measuring bulk carbon isotopes in isolation. The implication from this work is, as the authors suggest, that carbon isotope values alone are not reliable for organic matter provenance studies, particularly in pre-Cenozoic sediments.

The possibility of fairly substantial variations in marine carbon isotope values resulting from variable photosynthetic fractionation is worth much closer investigation. Positive carbon isotope shifts in carbonates during the Cretaceous could represent increased oceanic productivity that uses up more of the lighter isotope¹⁰. The work of Arthur *et al.* could upset some of the widely accepted ideas for models of the carbon cycle. Clearly, more detailed work is required, but merely taking bulk carbon isotope values of organic matter in sediments in isolation is not going to help solve the problem. □

1. Arthur, M.A., Dean, W.E. & Claypool, G.E. *Nature* **315**, 216 (1985).
2. Sackett, W.M. *Mar. Geol.* **2**, 173 (1964).
3. Hedges, J.I. & Parker, P.L. *Geochim. cosmochim. Acta* **40**, 1019 (1976).
4. Espitalie, J. *et al. Rev. Inst. Français du Pétrole* **32**, 23 (1977).
5. Schoell, M. & Wellmer, F.W. *Nature* **290**, 696 (1981).
6. Hayes, J.M., Kaplan, I.R. & Wedeking, K.W. in *Earth's Early Biosphere* (ed. Schopf, J.W.) (Princeton Univ. Press, 1983).
7. Gilmour I., Gibson, E.K., Abell, P.I. & Pillinger C.T. *Terra Cognita* **5**, 145 (1985).
8. Deuser, J.M., Degens, E.T. & Guillard, R.R.L. *Geochim. cosmochim. Acta* **32**, 657 (1968).
9. Wong, W.W. & Sackett, W.M. *Geochim. cosmochim. Acta* **42**, 1809 (1978).
10. Weissert, H.J., McKenzie, J.A. & Channell, J.E.T. *Terra Cognita* **5**, 111 (1985).

I. Gilmour is in the Department of Earth Sciences, The Open University, Milton Keynes, Buckinghamshire MK7 6AA, UK.

Scientific creationism and rationality

from Michael A. Cavanaugh

Scientific creationism is a social movement of rationalists — amateur philosophers of science — whose claims upon science policy are nonetheless wholly unreasonable. This thesis may not endear the author either to rationalists or creationists, but it may illumine the movement's behaviour and the sources of its wider appeal.

ORDINARY language contrasts the rational and the social. Individuals either pursue the truth or we pursue our interests; either we have good reasons or we are swayed by others — peers, experts or traditions. On another point of view, derived from the sociologists Durkheim and Weber, society is defined by its shared rationality. Social structures and divisions of labour can distribute, even augment, human reason (or impede it). From this standpoint, society as such is "knowledge-based; and two heads are better than one. This, if so, is so for any society.

Modern societies are knowledge-based in yet another way: they thrive upon mass education¹. Schooling has become indispensable for even ordinary occupational roles; fairly advanced educational credentials are demanded routinely as tickets of admission to the work force^{1,2}. The consequences are political and cultural as well as economic. Complex societies discover that they cannot dispense with technical expertise. And this is no less true (as in Iran and China in recent years) when the power of technicians and experts conflicts with traditional elites or newer populist authority^{3,4}.

Mass education

Mass education is oddly uneven education. It enlarges society's pool of intellectuals⁵, but also confers academic degrees upon people contemptuous of intellect and unfamiliar with the life of the mind. Mass education democratizes educational opportunity; it also stratifies the "new class" of the highly schooled and proliferates an intellectual underworld — Pluto's Republic, *pace* Peter Medawar.

So it is that late twentieth-century society simultaneously enjoys unprecedented levels of education and technical knowledge and suffers acute self-doubt and pessimism about the very value of human reason. "Once again," as Medawar⁶ has observed, "there is a rootlessness or ambivalence about philosophical thinking, as if the discovery or rediscovery of the insufficiency of reason had given a paradoxical validity to nonsense, and this gives us a special sympathy for the

dilemmas of the seventeenth century."

How apt, as our era goes through a debate more appropriate to the age of Bacon, Newton and Descartes (or perhaps, of Thomas Aquinas). Claiming support from the scientific community, highly schooled fundamentalist Protestants propose teaching a "creation model" as an alternative to evolution. Since their heyday during 1980–82, such claims and proposals have not gone undisputed. To its opponents, creationism appears as Luddite anti-intellectualism⁷; as pseudoscience⁸; as a deviant science movement⁹; and as an American aberration (rooted in the rural South)⁷. Its advocates are portrayed as equally uninformed about genuine science as about genuine religion¹⁰; or as "space-age fundamentalists"¹¹, religious traditionalists as inconsistent as the "republican" *mullahs* denouncing Western

...almost all contemporary scientific creationists claim bachelor's degrees...forty to sixty per cent in some scientific or technical field...

materialism into the uplifted microphones of mass-produced tape recorders.

Religious fundamentalism is nothing if not a protest against modern secular society¹². Yet to regard scientific creationism simply as marginal to that society is misleading. On the contrary, creationism is not the anti-intellectualism of those who have just gotten shoes; it is the counter-intellectualism of modern offspring (who understand well the rhetoric of modern political pluralism). Consider educational experiences. Unlike their fore-runners of the 1920s, contemporary scientific creationists are the beneficiaries of mass educational expansion. Almost all claim bachelor's degrees, an estimated 40–60 per cent in some scientific or technical field. Around 10–25 per cent are trained in applied areas, particularly engineering, 20–30 per cent in physical science; and a few (5–10 per cent) in the life sciences, the most relevant for an anti-Darwinian movement. (Some are now completing graduate work pseudonymously.) Many claim PhDs from accredited schools, even major universities. (Some claims have been disputed¹³.) Compare this with the average US citizen's 14.2 years of schooling.

In social location, the movement is not confined either to rural margins or to the American South. It thrives wherever Protestant natural theology has spread, mainly throughout the former British Empire (for example, North America, Australasia, South Africa, India, the United Kingdom itself), concentrating in North America (primarily in expanding urban areas, in longstanding fundamentalist enclaves in midwestern and western states and provinces but not in the southern United States)¹⁴.

The geographical and occupational distribution of creationists illustrates how fundamentalism is expanding beyond its marginal enclaves. Erstwhile fundamentalists separated themselves into subcultures with unaccredited theologically-based schools¹². Like their secular contemporaries, today's fundamentalists seek technical credentials for occupational mobility; thus they must neutralize the worldly blandishments from which their forebears simply fled¹⁵. *Pace* Snow's "two cultures"¹⁶, young fundamentalists desire (and their parents encourage) technical training with minimal humanistic education. Thus the Reverend Mr Jerry Falwell (the leader of the Moral Majority) and Mrs Phyllis Schlafly (the lawyer opposed to expanding women's rights) wish their children to become engineers. Why? Because, the parents reason^{17,18}, unlike law (not to mention literature, or philosophy), engineering devises simple solutions to practical problems, without seriously challenging the intellectual traditions of any subculture (religious, political, or otherwise). Questionable though their analysis of engineering may be^{19,20}, this does suggest why technical training appeals to fundamentalists — Christian, Jewish, Muslim — and others (in developed and developing nations alike) seeking to avoid the choices underlying modern science and technology.

Like Islamic republicanism, scientific creationism is not simply backwardness; both are cautious bargains with the devil, modernity. Although creationism's conceptual resources betray a seventeenth-century provenance, its causes (and appeal) are discernibly more recent; and it is not entirely foreign to the normal culture of science. Why does the knowledge-based society respond to creationism *redivivus*,

Michael A. Cavanaugh is at the Department of Sociology, Temple University, Philadelphia, Pennsylvania 19122, USA.

rather than Taoist alchemy or Babylonian cosmology? And why are there so many Islamic republicans but no Islamic creationists? In brief, because the West has a unique history of progressively testing key religious myths for purely secular validity⁴, and creationism was tested and found wanting. Because of this legacy, there are some things creationism definitely is not. For instance, it is not preindustrial Luddite irrationalism. It is not a deviant science movement like plate tectonics once was. Nor is it pseudoscience in precisely the way that "psychic archaeology", phrenology or the green cheese theory of the Moon are. Unlike them, creationism once enjoyed standing in the history of science.

Scientific method

Robust in Newton's day, creationism degenerated²¹, and no longer informs any active progressing scientific research tradition^{22,23}. To survey the main social and intellectual hallmarks that identify science is to discover what creationism lacks. First, knowledge is acquired through conjectures and refutations in a research process²⁴. Research is institutionalized in the various scientific professions, which select and certify practitioners, and is communicated in the various journals. Therein research is subjected, first and foremost, to the judgement (and reward) of specialist peers²⁵. Subsequent public acclaim or academic standing depends on this judgement.

Second, whence the coherence of science? It cannot be founded upon tenets, which change. Any single tenet may be false; most (the set of all scientific theories minus our present ones) are false. Moreover, the foundationalist search for substantive bedrock is now widely acknowledged to have failed²⁶. Science acquires coherence, rather, through its procedural commitment to the integrity, reliability and professional governance of scientific theory-testing (and teaching) over the long haul²⁷. In other words, modern science rests (like modern law) upon long-term "orthopraxy" (the integrity of method, institutionalized in professions) rather than any short-term orthodoxy of individually trustworthy tenets.

Accordingly, the unit of knowledge acquisition is the theoretical community rather than the individual scientist; thus "science" develops as the distributed property of communities of theory (research traditions), not the consolidated property of individual genius — much less, of what Everyman observes in an untutored way. (On this view, theory is not the luxurious icing on the scientific cake, but the very cake itself. Science is nothing other than the history of its theories, since a method-independent science is impossible, and the subjects of this history are the scientific collectivities that produce and change theories^{28,23}.)

Third, contemporary science is organized in certain ways. It is "big science"²⁹.

Science is now conducted typically by career scientists within large complex organizations (rather than as the loosely organized hobby of gentleman amateurs). Increased size refines divisions of labour; consequently, "science" becomes even less the concentrated property of individuals. Each may differ greatly in understanding, talent and skill, yet all may contribute (provided their labour is divided cleverly enough) to the limits of their diverse gifts. Big science is internally "democratized", which permits the fullest use of even modest talent^{6,30}. (This is, perhaps, the chief difference between organized science and organized crime.)

The organizational factor makes for a complex relationship of control between science and society. On the one hand, though the typical scientist is not Einstein, he will enjoy some expert divergence from commonsense not shared by every citizen. On the other hand, science and commonsense are not totally antithetical, for in a knowledge-based society, commonsense is increasingly tutored by "packed-down science"³¹. Thus the man on the modern street "sees" the roundness of the Earth, and his common sense "knows" that the heart is not the seat of emotion and that werewolves do not exist. Such "conventional wisdom" is primarily a fact, not of individual psychology, but of membership in a certain kind of society. The organized process of gaining, and warranting, knowledge differs from the ability to use it in packed-down form, or the ability of Everyman individually to validate what he knows.

It is the division of expert labour among a network of complex professional organizations that distinguishes contemporary science from such other enterprises as arm-chair scepticism, interest-group politics and even the "little science" practised by amateurs (in the best sense) over the centuries past. If such are the hallmarks of science,

Big science is internally democratized, permitting the fullest use of even modest talents. This is perhaps the chief difference between organized science and organized crime.

then it is clear why creationism is not a scientific movement, not even a deviant one. As a rule (with few exceptions), its adherents do not undertake to conjecture and refute through empirical research³². They circumvent the normal confines of professions and journals, going "over the heads" of peer researchers and reviewers in favour of other forums such as the popular press, textbook selection committees, legislatures and courts of law^{12,33}. Popular acclaim, not expert peer review, is their ultimate court of appeal.

While it is true that (in recent decades) creationists have not tried to outlaw evolution, they have sought to establish anti-evolutionary tenets by legal fiat. And characteristically, the campaign for lasting

orthodox tenets advances by a straightforwardly empiricist rejection of evolutionary theory, as if theory meant imperfect fact — "part of a hierarchy of confidence running downhill from fact to theory to hypothesis to guess"³⁴ — something in dire need of extra-scientific correction.

Organized scepticism

Thus, if science is inseparable from self-governing professions, the procedures they institutionalize and (perhaps most importantly) the theories they conjecture and test, then scientific creationism does not resemble science. It requires nothing more arcane than well-known philosophical and sociological demarcations to understand why public policy justly may disregard creationist demands to produce science through political rather than scientific processes.

Faced with this assessment, a sophisticated creationist might invoke his scepticism and empiricism. He might assert that creationism is highly sceptical about tautology in evolutionary theory³⁵, whereas evolution requires mere faith in insubstantial theories. Does this count? Old chestnuts about tautology, verifiability and naive falsification^{36,37} aside, the answer must be no. Why? First, the process of criticism in contemporary science deploys the norm of "organized scepticism"³⁰ seeking cumulative growth in knowledge. Creationist scepticism occurs sporadically outside organized science and seeks selectively to prune whatever accumulated knowledge offends extra-scientific tastes.

Second, categorical "scepticism" masks an underlying foundationalism (religious fundamentalism being the limiting case). Categorical or naive doubt is not scepticism. For doubt to be intellectually credible, the specific occasions and ends of doubt make all the difference. Creationists understand this intuition, for they, too, doubt selectively; as members of the knowledge-based society, their views retain much presupposed common-sensical packed-down science (that the Earth is round; that the heart is a pump; that men do not change into werewolves; that fossils are traces of extinct forms^{38,39}). So, during fieldwork¹⁴, one informant complained about a long-distance telephone call to a fellow creationist (one of the few who is also a Flat-Earther): "I do not intend to spend \$1 per minute discussing nonsense!" Like most creationists, he doubts — but only those views (scientific, or superstitious) that countermand the foundational tenets of fundamentalist Protestantism.

Creationism is radically against method. Procedure is disregarded in favour of direct access to a science-independent, oracular, God's-eye view of nature (literally so). So why not ignore normal research channels and limit oneself, as creationists do, in the worst tradition of academic scholasticism, to *post factum* pot-shotting? Likewise creationists invoke the rhetoric of scepticism and empiricism. They will assert that such-

and-such an evolutionary finding cannot be replicated in a laboratory⁴⁰, or is not the sort of thing publicly observable by the common man⁴¹, or would not meet lawyers' tests of evidence³⁵, and so is scientifically invalid. But these 'sceptical' arguments are marshalled in the interests of a submerged theological agenda⁴². Thus empiricism and scepticism, ordinarily laudable scientific hallmarks, are directed toward "doxological science"⁴³, which is not. Like Cartesians, creationists doubt only so as to shore up science-independent foundational certitude; unlike Cartesians, a preterhuman card waits up their sleeves. Moreover, by appealing to individualistic common-sense tests in the court of public opinion, creationists simply misapprehend the ineliminably distributed property of science and scientific theory change. (One of history's little jokes is that the very principled distrust of structured authority and tradition that wed seventeenth-century Protestant rationalism to little science⁴⁴ now divorces it from big science.)

Science and parascience

Habitually circumventing the social and intellectual structure of modern science, now inseparably linked by big science as never before, weighs decisively against creationism's scientific standing. If not science, then what? Creationism is neither deviant science nor simply pseudoscience. It is best appreciated as a specialized *parascientific* movement: one whose members operate outside professional channels of research while still concerned with the criticism, use

...creationist parascientists run the gamut from Doubting Thomists to Popper-choppers.

and dissemination of scientific findings. (Philosophers of science are another such group, likewise, other science analysts, scientific popularizers and amateur naturalists such as bird watchers. For various reasons, many modern enterprises are parascientifically tinged; and now even religions typically vie for legitimacy consonant with the cultural authority of science, rather than asserting independent bases of authority⁴⁵.)

Behaviourally, some creationists are amateur naturalists, fossil-hunters for example (and perhaps there are more creationists in North America than in Britain just because there are fewer bird watchers). But on the whole, creationists are amateur, anachronistic philosophers of science, acting (unlike either working scientists or professional philosophers of science) to alter the content of scientific knowledge piecemeal through plebiscite and lawsuit rather than systematically through influencing professional debates and research activities. They embrace a degenerate research tradition. Their classical mode combines a scholastic preference for oracular science-independent knowledge with a quaintly early-modern mixture of Baconian empiricism and

Scottish common-sense realism^{12,43}. But lately, some creationists have begun to speak of models, paradigms and falsification. Thus creationist parascientists run the gamut from Doubting Thomists to Popper-choppers.

Amateur philosophy

Scientific creationists suffer a philosophy of science at odds with contemporary philosophy of science as well as with contemporary scientific practice. Their departure from science's professions, procedures and organized scepticism in favour of extra-scientific correction of scientific knowledge is at once principled and opportunistic. It is shaped by the scholastic principles of doubting Thomism, which enjoin opportunistic pruning of whatever contravenes orthodox revelation; quite inimical resources (even Popperian philosophy) will be mobilized as opportunity arises.

The terms "philosophical" and "intellectual" here confer no normative dignity, but, as readers of *Nature* will know, the view that creationism is latter-day scholasticism has been disputed. Criticizing Marsden (its main proponent^{12,46}), Jukes⁴⁷ asserts that the creationist dispute "is not a polite discussion of differences in philosophy", but a fight over the worst sort of obscurantism. Would that politeness preclude obscurantism; in my research experience, creationists are on the whole polite, indeed amiable. But personality aside, to debate creationism is to quit Plato's for Pluto's Republic, and there is much evidence of Jukes' general view.

As a movement, creationism routinely flouts fundamental intellectual norms (obligations, for example, to earn expertise rather than flaunting credentials; to scrutinize allies as rigorously as opponents; to detect and punish fraud among peers; to meet critic's counterarguments; to acknowledge criticism and retract assertions if the criticism cannot be met^{7,37,47,49}). This granted, has Jukes claimed any more than that creationists are ungentlemanly intellectuals and bad philosophers? Marsden's contentions that fundamentalism is not anti-intellectualism but a distinct kind of modern intellectual movement, and that creationists are fundamentally Protestant scholastics, stand on their scholarly merits. Jukes has not refuted them; it is not clear that he has even contradicted them. (It is otherwise with Marsden's view that evolutionary theory is usefully understood as "anti-supernaturalistic mythology"⁴⁶, to which Jukes also objects.)

To be sure, the entire creationist campaign is populated with figures such as the "textbook watchers"¹¹ who are textbook cases of anti-intellectualism⁴⁸. But members of movement organizations tend to be counter-intellectuals, and the movement as a whole relies on them to produce its ideology. Religious intellectuals approach science in various ways¹⁴, the chief options being scholasticism and Averroism. These are, respectively, the view that

natural knowledge and revealed knowledge mutually corroborate (classically, that the self-same Author wrote the 'Book of Nature' and the 'Book of Scripture', and the view (typically post-kantian) that natural knowledge and revelation treat different domains. For scholasticism, religion is like science, in several ways. First, theology is commensurable with science (whereas, for Averroism, the two are radically incommensurable, and religion is not just lame science). Second, for scholastics, both science and theology are systems of propositional truths; deny any single truth, and the whole system is weakened, perhaps fatally.

Scholasticism inclines towards scientific realism (against conventionalism) and enjoins extra-scientific correction. Creationists are scholastics. Hence they will produce lists⁵⁰ of real truths about nature, allegedly anticipated by Biblical writers and only later verified by science. Hence the unspoken premise of creationist rhetoric: "Even (name any authoritative evolutionist) admits that X..." One cannot "admit" novel knowledge, only what every Schoolman already knows. When scientific experts are found to "admit" X, Y, and Z in this manner, mere human method-beholden science is corrected in the light of superior knowledge.

How is it possible to sustain such a view? Oracular science, like oracular law, may be feasible within delimited subcultures, but it is an impossible luxury within complex societies. There, Averroism secures civil and religious peace; and even if the scientific substance of creationism had not been discredited (as it was, manifestly, during the darwinian revolution), a hearing for such a secretarian epistemology must be sought in vain within the central-societal, cosmopolitan media of communication.

Sociologically, the short answer^{12,43,51} is that sectarian Protestantism (aided especially by the segmented social structure of North America) preserved older scholastic philosophy in insulated subcultural en-

Creationists are scholastics... they will produce lists of real truths about nature allegedly anticipated by Biblical writers and only later verified by science.

claves with their own schools, presses and other (peripheral) media of communications. They thus bypassed entirely Averroism and the kantian and darwinian revolutions in the more cosmopolitan media.

Scholastic "proto-postivism"²¹ regards the ordinary untutored observer as the unit of knowledge acquisition. Naive empiricism is applied to a naively real world in quest of inductively established certainty; 'proof' is demanded equally of religious and scientific propositions. Thus Arthur Pierson, in *Many Infallible Proofs. The Evidence of Christianity* (1886), could say, "Nothing," either scientific or religious,

"is to be accepted unless based in good evidence," so that if the Bible is read "in a truly impartial and scientific spirit," pious "Thomistic" doubt will dispel infidel doubt¹². Religion is rational, like science; so science is like theology, comprised of certain evident propositions about a real universe, not of more-or-less probable, conventional, theory-laden conjectures.

The same pre-kantian spirit of doxological science, evident in such members of the "parson naturalist" tradition as John Ray, Gilbert White and Jonathan Edwards, and also in the writings of Newton and fellow members of the Royal Society^{52,53}, continues. It echoes in Paley's memorable *Natural Theology*⁵⁴ as well as in forgotten fustian titles⁵⁵⁻⁶⁰ and their successors⁶¹⁻⁶³. Its arguments were merely rephrased when Mr Ivan Stonehocker (President, Creation Science Association of Alberta) responded⁶⁴ to the claim by the provincial minister of education that the scientific community, not public education, determined the validity of scientific claims:

The scientific community accepts on faith the assumption that the universe and life on earth had a natural beginning. There is no evidence or proof for that position — it is simply a philosophy, nothing more. Science can only properly deal with events which are observable, repeatable and testable... (thus) all discussion of evolution should be turfed out of all courses and all textbooks.

Much the same happened when, on US television, creationist the Reverend Mr James Robinson gleefully accused evolutionist Carl Sagan of relying on mere faith in unproven theories. Fundamentalism turns out to be some kind of arch-rationalism.

Compare Australian creationist Mr A.W. Mehler on punctuated equilibrium: "...unless you believe in natural miracles... the hard facts (*sic*) of science are in strong disagreement with suggested mechanisms to support the new evolution"⁶⁵. Or compare Professor H. Enoch, MA, FZS, retired chairman of the Zoology Department, University of Madras, and now a Vice-President of the Creation Science Movement: "Careful reading of the Scriptures, with an open mind, will reveal that no established scientific fact contradicts the story of creation as described in the first chapter of the Bible"⁶⁶.

...there are many men who, though knowing absolutely nothing of the subject with which they are dealing, wish, nevertheless, to damage the author of some view with which they think fit to disagree. What they do, then, is not to go and learn something about the subject, ... (but) wind up saying that: "After all, you know, the principles and methods of this author are totally opposed to the canons of the Baconian philosophy."

Thus, Thomas H. Huxley⁶⁷ on some of his contemporaries — and some of ours. If scientific knowledge (or selected offending parts of it) cannot be "proven" in a laboratory, or rests on theoretical conjecture, or falls short of inductive certainty from com-

mon-sense observation of the facts, then away with it; it is not science. As younger fundamentalists join their contemporaries in mass education, they are exposed to post-nineteenth-century philosophy, and find it just as easy to abuse their opponents with Popperian post-positivism as with Baconian protopositivism⁶⁸.

This is not Popperianism, it is Popper-chopping. Popper is chopped in two ways. First, anomalous or difficult data are held to falsify decisively. Second, Popper's erstwhile scepticism about Darwinism as a "metaphysical research programme"⁶⁹ is invoked. The former, "naïve falsificationism"³⁷, forgets that any theory will be underdetermined by the data available, so that knock-down falsification is quite uncommon. Second, although Popper said "metaphysical", he also circumspectly said "research programme". Popper has, in any case, since retracted this criticism^{70,71}. Thus creationist Popper-chopping is (at best) disingenuous⁷².

Opportunity structure

Influence abhors a vacuum, and opportunists require 'opportunity structures'⁷³. So why are creationists so influential? Not through sheer numbers; the movement's core of adherents is small, and its kernel (actual members of scientific creationist organizations) numbers no more than several thousand worldwide. Influence amplifies within an opportunity structure whereby (1) the core uses (2) a protective belt of mainstream intellectuals (willing and

Perhaps the disproportion of physicists and engineers in the creationist movement is no accident... physical scientists are professionally disposed to puzzle over central biological concepts.

unwilling) plus (3) the contemporary climate of public opinion and scientific understanding.

The protective belt consists of draftees and volunteers. With sufficiently loose standards of argument, the pool of draftees approaches infinity; reading creationists, "one would think that the main spokesman for the fundamentalist cause are Ernst Mayr, G.G. Simpson, Theodosius Dobzhansky and Stephen Jay Gould!"⁷⁷ Eldredge and Gould have been drafted (protests^{34,74} unheeded) because punctuated equilibrium⁷⁵ challenges Darwin's own gradualist view of the tempo and mode of evolution; and whereas doubting Thomism thinks science requires foundational certainty, in principle it cannot distinguish typical intra-scientific dissent from outright repudiation of evolution. Thus creationist Mehler⁶⁵: "Each time the differing evolutionist factions attack each other, all the profit goes to the creationists who can reap the best from both worlds." Likewise, Popper's scepticism is noted, but neither his circumscription nor subsequent retraction.

Such a draft is difficult to dodge; some

do not try, although there are degrees of volunteering. Perhaps the disproportion of physicists and engineers in the creationist movement (and their resurrection of misapplied probability arguments^{37,76,77}) is not accidental; physical scientists are professionally disposed to puzzle over central biological concepts (for example, that individuals in a species are not identical in the way that atoms of an element are; that populations and their dynamics, not individuals, are the units of selection; that consequently "in complex, historically formed systems things occur that do not occur in inanimate systems"⁷⁸). Beyond intra-scientific misunderstanding, lawyer Norman MacBeth³⁵ mistakes scientific for legal truth-testing. However, the track record of a research programme carries over from case to case (whereas that of contesting lawyers does not); and the analogy, evolution: creationism:: American prosecutorial burden of proof: defendant's presumptive innocence, is unsound.

At what point does confusion pass into wilfulness? Liberal theologian Huston Smith thinks⁷⁹ that creationism does public service in policing "the scientific establishment" lest mechanism and reductionism unweave the rainbow. Paul Feyereabend (thoroughly "against method"⁸⁰) proclaims: "Three cheers to the fundamentalists in California who succeeded in having a dogmatic formulation of the theory of evolution removed from the textbooks and an account of Genesis included"⁸¹. (His immediate disclaimer that a creationist establishment would also be dogmatic may strike some as small consolation.) And what of a palaeontologist, the Sorbonne's Pierre Grasse⁸², whose nationalist disdain for "daydreaming" across the Channel produces: "The probability of dust carried by the wind reproducing Durer's 'Melancholia' is less infinitesimal than the probability of copy errors in the DNA molecules leading to the formation of the eye..."? Fred Hoyle propagates⁸³ this same elementary misunderstanding, now a creationist mainstay^{84,85}. Likewise the claim that natural selection is tautologous in which Hoyle is joined by palaeontologist Colin Patterson of the British Museum (NH). Creationists take much aid and comfort here. A 1981 talk in which Patterson likened evolutionary theory to religious

Fred Hoyle propagates this same elementary misunderstanding, now a creationist mainstay.

dogmatism, and said that "evolution not only conveys no knowledge but seems somehow to convey anti-knowledge", was transcribed and circulated (over Patterson's protest⁸⁶) by creationist engineer Mr Luther Sunderland. This is odd, since Patterson also dubs creationism "anti-knowledge"; but he also asserts a baconian view of evidence and theory-testing. On "Proof and Disproof" (*sic*)⁸⁷, Patterson commends Darwin's misleading claim that

he was prepared "to give up any hypothesis . . . as soon as the facts are shown to be opposed to it" and warns: "evolutionary theory is not testable in the same way as a theory in physics, or chemistry, or genetics, by experiments designed to falsify it." Finally, there is the matter of transformed cladism (which Patterson⁸⁸ champions, along with various recent contributions to *Systematic Zoology*). Its goal of theory-free taxonomy is debateable⁸⁹, but its appeal for Doubting Thomists is understandable.

Howbeit willing or unwilling, the protective belt is useful only within a particular climate of scientific understanding. Much ink has been spilt over the rediscovery in recent decades of the insufficiency of reason, and of other curiosities of post-industrial society such as the fact that many people both ride the Clapham omnibus and consult their horoscopes. Yet caution might be urged in interpreting research evidence in favour of pervasive anti-rationalism. For example, one poll taken late in 1981⁹⁰ (at the height of the furor) was widely reported as showing that 86 per cent of Americans favoured creationism. What it actually showed was that only 10 per cent believed in creationism, while 76 per cent favoured teaching both creation and evolution. A more recent survey of science students at Ohio State University corroborated, with 80 per cent in favour of both in science courses, although students with increased

education in biology were less likely to concur⁹¹. This suggests that it was not creationism, but belief in fair play (or perhaps the belief that both are "only" theories, and thus on a par) that is at work.

Clearly, the creationists pitch their public appeal to a widespread cultural belief in fair play and equal time for the underdog¹¹. And their campaign must attract support, perennially, to the extent that it is able to tap perennial conditions such as "Pluto's Republic" fed by mass education, populist distrust of professional politics in democracies, a folk-epistemology of common-sense empiricism, and simply poor scientific education (of the sort that especially afflicts America, in part because of the erosion of biological curricula by creationism). In other words, the "success" of creationism may be an artifact of the "one-eyed man effect"⁹², in which fervently communicated belief fills a vacuum simply in default of any better-defended or better communicated contender. On the other hand, creationism itself is parasitic on the distinctively Western culture of science, including the packed-down science characteristic of modern common-sense knowledge. Thus, not surprisingly, there is some evidence from the counter-mobilization of anti-creationists^{93,94} that (just because creationism is likely to appeal to those with an interest in and limited knowledge of science) members of the public initially

disposed to entertain creationism will change their minds when confronted with a well-argued case for evolution.

Whither creationism?

If the events of 1980-82 appeared as a flash in the pan, the social forces conducive to the creationist movement are perennial. It may disappear from time to time below the threshold of public perception. It has not thereby gone away; though "monkey trials" may not make the headlines, creationists are still at work. Upgrading the climate, and deflating the protective belt, might curtail the disproportionate influence of the movement's core; and, if it does thrive on an outmoded philosophy of science, then including the history, philosophy, and sociology of science as an integral part of science education might exert significant deflationary pressure. Some might regard attention to the climate of "conventional wisdom" as a mere hobby or distraction from real science. However, although the conventional wisdom may be conventional, it may also embody some wisdom and produce some consequences for the support of science; and the scientific community might well ask if concerted attention now is not a small price to pay in order to avoid future generations who, having passed through higher education, agree with that 25 per cent of Ohio State students who believe that evolution is just bunk⁹¹ — "even the scientists say so".

- Holzner, B. & Marx, J. *Knowledge Applications* (Allyn and Bacon, Boston, 1979).
- Collins, R. *The Credential Society* (Academic, New York, 1979).
- Gouldner, A. *The Dialectic of Ideology and Technology* (Seabury, New York, 1976).
- Gellner, E. *Legitimation of Belief* (Cambridge University Press, 1973).
- Gouldner, A. *The Future of Intellectuals and the Rise of the New Class* (Oxford, New York, 1979).
- Medawar, P. *Pluto's Republic* (Oxford, New York, 1982).
- Ruse, M. *New Scientist*, 94, 586-591 (1982).
- Cloud, P. *Humanist* 37, 6-15 (1977).
- Barker, E. in *On the Margins of Science* (ed. Wallis, R.) 179-200 (Keele, Hanley, 1979).
- Gilkey, L. in *Is God a Creationist?* (ed. Frye, R.) 56-57 (Scribner, New York, 1984).
- Nelkin, D. *The Creationist Controversy* (Norton, New York, 1982).
- Marsden, G. *Fundamentalism and American Culture* (Oxford, New York, 1980).
- Root-Bernstein, R. *Science and Creationism* (ed. Montagu, A.) 64-94 (Oxford, New York, 1984).
- Cavanaugh, M. thesis, Univ. Pittsburgh (1983).
- Hunter, J. *American Evangelicalism* (Rutgers, New Brunswick, 1983).
- Snow, C.P. *The Two Cultures and the Scientific Revolution* (Cambridge University Press, 1959).
- FitzGerald, F. *New Yorker*, 53-141 (18 May 1981).
- FitzGerald, F. *NY Rev. Books*, 19-26 (19 November 1981).
- Florman, S. *The Existential Pleasures of Engineering* (St. Martin's, New York, 1976).
- Patterson, J. *Proc. Iowa Acad. Sci.* 89, 55-58 (1982).
- Gillespie, N. *Charles Darwin and the Problem of Creation* (Chicago University Press, 1979).
- Lakatos, I. *The Methodology of Scientific Research Programmes* (Cambridge University Press, 1978).
- Laudan, L. *Progress and its Problems* (California, Berkeley, 1977).
- Popper, K. *Conjectures and Refutations* (Harper, New York, 1963).
- Merton, R. *The Sociology of Science* (Chicago University Press, 1973).
- Rorty, R. *Philosophy and the Mirror of Nature* (Princeton University Press, 1979).
- Popper, K. *The Logic of Scientific Discovery* (Harper, New York, 1959).
- Toulmin, S. *Human Understanding* (Chicago University Press, 1972).
- Price, D. deS. *Big Science, Little Science* (Columbia, New York, 1962).

- Merton, R. *New Scientist*, 12, 306-308 (1961).
- Price, D. deS. in *Coping with the Biomedical Literature* (ed. Warren, K.) 3-16 (Praeger, New York, 1981).
- Scott, E. & Cole, H. *Phi Delta Kappan*, 557-558 (April 1982).
- LaFollette, M. *Creationism, Science, and the Law* (Massachusetts Institute of Technology, Cambridge, 1983).
- Gould, S. *Hen's Teeth and Horse's Toes* (Norton, New York, 1983).
- MacBeth, N. *Darwin Retried* (Gambit, Boston, 1971).
- Williams, M. *Philosophy Sci.* 40, 518-537 (1973).
- Kitcher, P. *Abusing Science* (Massachusetts Institute of Technology, Cambridge, 1982).
- Brewster, E. *Creation* (Bobbs-Merrill, Indianapolis, 1927).
- Rudwick, M. *The Meaning of Fossils* (Science History Publications, New York, 1976).
- Moore, J.N. *Creation Re. Soc. Quarterly* 7, 105-116 (1970).
- Hall, M. & Hall, S. *The Truth* (Baker, Grand Rapids, 1975).
- Overton, W. *Science* 215, 934-943 (1982).
- Bozeman, T. *Protestants in an Age of Science* (Chapel Hill, North Carolina, 1977).
- Merton, R. *Science, Technology and Society in Seventeenth-century England* (Harper, New York, 1970).
- Barker, E. A. *Rev. Soc. Sci. Religion* 3, 79-103 (1979).
- Marsden, G. *Nature* 305, 571-574 (1983).
- Jukes, T. *Nature* 308, 398-400 (1984).
- Hofstadter, R. *Anti-intellectualism in American Life* (Harvard, Boston, 1963).
- Weber, G. *Creation/Evolution Newsletter*, 4 (July/August 1984).
- Stoner, P. *Science Speaks* (Moody, Chicago, 1958).
- Dillenberger, J. *Protestant Thought and Natural Science* (Doubleday, Garden City, 1960).
- Ray, J. *The Wisdom of God Manifested in the Works of His Creation* (1691).
- Boyle, R. *Christian Virtuoso, Shewing that by being addicted to Experimental Philosophy, a man is rather assisted than impeded to be a good Christian* (1690).
- Paley, W. *Natural Theology* (1802).
- Gosse, P. *Omphalos* (1857).
- Lord, D. *Geognosy, or the Facts and Principles of Geology against Theories* (1855).
- Rutherford, R. *Fables of Infidelity and Facts of Faith* (1875).
- Hodgman, S. *Moses and the Philosophers... Giving a View of the Universe, as Written by Moses, The Servant of God* (1884; 1841).
- Gridley, A. *The First Chapter of Genesis as Rock Foundation for Science and Religion* (1913).
- McCann, A. *God — or Gorilla? How the Monkey Theory of Evolution Exposes its Own Methods, Refutes its Own Principles, Denies its Own Inferences* (1922).
- Gish, D. *Evolution: The Fossils Say NO!* (Creation-Life, San

- Diego, 1978).
- Morris, J. *Tracking Those Incredible Dinosaurs & the People Who Knew Them* (Bethany House, Minneapolis, 1980).
- Morris, H. *Men of Science, Men of God* (Creation-Life, San Diego, 1982).
- Stonehocker, I. *Creation-Science Dialogue* 8, 5-6 (May 1981).
- Mehlert, A. *Bible-Science Newsletter* 22, 14 (December 1984).
- Enoch, H. *Evolution or Creation* (Evangelical Press, Welwyn, Herts, 1967).
- Huxley, T. *The Essence of T.H. Huxley* (Macmillan, London, 1967).
- Ruse, M. in *Science and Creationism* (ed. Montagu, A.) 311-342 (Oxford, New York, 1984).
- Popper, K. *The Unended Quest* (Fontana, London, 1976).
- Popper, K. *Dialectica* 32, 339-355 (1978).
- Popper, K. *New Scientist*, 87, 611 (1980).
- Root-Bernstein, R. *Science* 213, 1446-1449 (1981).
- Merton, R. *Social Theory and Social Structure* (Free Press, New York, 1968).
- Eldredge, N. *Monkey Business* (Washington Square, New York, 1982).
- Gould, S. & Eldredge, N. *Paleobiology* 3, 115-151 (1977).
- Moorhead, P. & Kaplan, M. *Mathematical Challenges to the Neo-Darwinian Interpretation of Evolution* (Wistar Institute, Philadelphia, 1967).
- Patterson, J. in *Evolutionists Confront Creationists* (eds Awbrey, F. & Thwaites, W.) 132-152 (AAS, Pacific Division, San Francisco, 1984).
- Lewin, R. *Science* 216, 718-720 (1982).
- Smith, H. *Beyond the Post-Modern Mind* (Crossroads, New York, 1982).
- Feyerabend, P. *Against Method* (New Left, London 1972).
- Feyerabend, P. in *Scientific Revolutions* (ed. Hacking, I.) 156-167 (Oxford University Press, 1981).
- Grasse, P. *The Evolution of Living Organisms* (Academic, New York, 1974).
- Hoyle, F. *The Intelligent Universe* (Holt, Rinehart & Winston, New York, 1984).
- Halstead, B. *New Scientist*, 100, 940-941 (1983).
- Cherfas, J. *New Scientist*, 98, 486-487 (1983).
- Creation/Evolution Newsletter, 4, 4-5 (July/August 1984).
- Patterson, C. *Evolution* (British Museum/Cornell, 1978).
- Patterson, C. *New Scientist*, 94, 303-306 (1982).
- Mayr, E. *Science* 214, 510-516 (1981).
- NY Times, L-4, 18 November 1981.
- Fuerst, P. *Ohio J. Sci.* 84, 218-228 (1984).
- Cavanaugh, M. *Phil. Soc. Sci.* (in the press).
- Awbrey, F. & W. Thwaites. "A Two-Model Creation vs. Evolution Course at San Diego State University," typescript.
- Milne, D. *Am. Bio. Teacher* 43, 235-266 (1981).

Evolution of tumours and the impact of molecular oncology

George Klein & Eva Klein

Department of Tumor Biology, Karolinska Institute, Box 60 400, S-104 01 Stockholm, Sweden

It is generally accepted that tumours arise through the accumulation of several changes affecting the control of cell growth. Recent advances in molecular biology have made it possible to define some of these changes in molecular terms and to trace the steps by which certain tumours evolve.

FEW would contest the statement that the division and differentiation of somatic cells in higher organisms are regulated by multiple controls. Because cancer develops by the emancipation of a single cell from host controls, it probably arises in several steps. This has been confirmed in a number of ways. Statistical analyses of age-incidence curves have suggested three to four successive mutation-like changes for leukaemias and six to seven for carcinomas (see refs 1, 2 for review). This must be considered the minimum, rather than the actual number, as only the rate-limiting steps will be detected by statistical analysis.

Histological, cytogenetic and experimental analyses have also confirmed that the natural history of spontaneously occurring human and animal cancers is usually a multistep process, based on the successive emergence of new subclones that replace their predecessors as a result of some selective growth advantage³⁻⁵. The only known exceptions are found in experimental viral oncology. Some of the retrovirus-transduced oncogenes (for example, *v-src*) can cause tumours in a single step. However, as demonstrated recently by Temin⁶, serial passage *in vivo* in the laboratory puts the *v-onc* genes carried by the directly transforming RNA tumour viruses under selective pressure. Multiple genetic changes that favour transforming and/or tumorigenic activity can accumulate in a single oncogene. The same principle is illustrated even more dramatically by the discovery of an increasing number of retroviruses carrying two unlinked oncogenes that make different products with complementary transforming effects. The natural process of spontaneous tumour development is quite different. Selection acts on the cell, not on a virus. This favours single changes in multiple genes.

Foulds^{3,4} was the first to analyse this process of 'tumour progression'⁷. He emphasized that the neoplastic phenotype is composed of several 'unit characteristics' capable of wide variation and able to reassort independently of each other. Thus, the same phenotypic endpoint can be reached through several alternative pathways⁴. Each progression step may reflect the activation, mutation or loss of different genes. Molecular biology has opened the door towards their definition. At least three major functional groups can be discerned already. The oncogenes represent the first group (for review, see ref. 8). They seem to act dominantly in the sense that they can induce transformation when transferred to cultured cells. The best-known example of this group of genes is the *ras* family, in which a single point mutation can confer tumorigenicity. It is, however, unclear whether mutant *ras* genes act as simple determinants *in vivo*. For example, in tumours from human patients the normal germline *ras* allele is often lost⁹.

Moreover, there is evidence for a second group of genes, the 'tumour suppressors' or 'anti-oncogenes', that may have to be deleted or functionally incapacitated before a tumour can arise. The most thoroughly analysed example of such a gene is *rb-1*, both alleles of which are lost in retinoblastoma tumour cells¹⁰⁻¹². It has been suggested that the *rb-1* gene may have a normal

regulatory function, possibly in the induction of an essential step in the terminal differentiation of the tissue^{11,13-16}. Some other neoplasms, such as Wilms' tumour, familial renal cell carcinoma, neuroblastoma and small-cell carcinoma of the lung (SCLC), seem similarly to require the loss of both alleles of a single gene. This evidence for recessive anti-oncogenes is consistent with much earlier reports of the suppression of tumorigenic behaviour in hybrids derived from the fusion of malignant with normal cells¹⁷⁻²⁰. Cytogenetic analysis of such hybrids and their high malignant segregants suggests the existence of different suppressor genes on different chromosomes²¹⁻²⁴. There is now evidence that the transforming effect of different retroviral oncogenes can be counteracted by different suppressor genes. Recently, revertants *in vitro* of Kirsten-murine sarcoma virus-transformed fibroblasts have been found to suppress *ras*, *fes* and *src*-transformed partner cells, but not cells transformed by *mos*, *fms* and *sis*, after somatic hybridization²⁵. Eventually, the suppressor genes may turn out to be as diverse as the oncogenes, if not more so.

The third group of genes that influence neoplastic behaviour may be designated modulators. They do not on their own transform normal into neoplastic cells, but they modify their spread in the organism. They probably constitute a large and heterogeneous group. For example, metastatic spread may be influenced by the genes of the major histocompatibility complex (MHC)²⁶⁻²⁹ and invasiveness by those controlling proteolytic and homing mechanisms^{30,31}; cellular resistance to immune rejection^{32,33} may also play a part. Here we shall discuss the way in which some cancers may develop by a series of different steps. We have selected cases in which individual steps have been analysed in genetic and/or molecular terms.

Burkitt's lymphoma and mouse plasmacytomas

The Epstein-Barr virus (EBV)-carrying African or high endemic form of Burkitt's lymphoma (BL) provides an interesting example of a human tumour that develops in several steps. The tumours are derived from cells of the B-lymphocyte lineage that is responsible for the production of immunoglobulin. At least three different genetic systems may be involved. The first is of DNA-viral rather than of cellular origin. EBV is a highly transforming agent for human B lymphocytes. Epidemiological evidence has shown that an early EBV infection of African children at a relatively high multiplicity contributes to the causation of the tumour³⁴. This is corroborated by the discovery that 97% of African Burkitt lymphomas carry multiple copies of the EBV genome in all their cells³⁵.

The second genetic system is of cellular origin, activated by chromosomal translocations characteristic of Burkitt lymphomas. With the exception of a single atypical BL-derived line³⁶, all Burkitt lymphomas so far studied have been found to carry one of three specific translocations. This applies not only to the EBV-carrying African form but also to the sporadic

Table 1 Gene interactions in the development of Burkitt's lymphoma

Oncogene or transforming gene	Documented effect	Hypothetical effect	
		<i>In vitro</i>	<i>In vivo</i>
EBV (Three active regions in transformed cells)	B-cell activation and immortalization <i>in vitro</i> . Activation of BCGF production. Synthesis of EBNA and LYDMA.	Autocrine stimulation. BCGF dependence, also reflected by clumping and low agarose clonability. Limited freezing of maturation; some terminal differentiation does occur or can be induced.	Creates latently infected B cells, dependent on and responsive to B-cell growth factor and susceptible to immune control.
Activated <i>myc</i>	Juxtaposition to an immunoglobulin locus by chromosomal translocation leads to constitutive <i>myc</i> expression.	Ability for BCGF independent growth, with or without maintained responsiveness, also reflected by high agarose clonability and growth in single cell suspension. Switched on <i>myc</i> drives cell division 'from within'?	Decreased dependence on positive growth signals from the outside. Resistance to differentiation induction?
Activated <i>ras</i> and/or <i>Blym</i> ?	Transformation of NIH 3T3 fibroblasts.	Changed growth factor or differentiation factor receptors?	May act in complementary or synergistic fashion with activated <i>myc</i> .

* Activation of these genes has only been demonstrated in some long-established *in vitro* lines.

non-endemic cases that occur all over the world. The translocations act by juxtaposing *c-myc* to one of the three immunoglobulin loci, normally active in B lymphocytes, with the result that the oncogene is constitutively activated (see ref. 37 for review). It is possible that a third oncogene system may be involved as well: it has been reported that NIH 3T3 fibroblasts can be transformed with DNA from BL-derived sequences that are related neither to EBV nor to *myc*. The identity of the transforming DNA is currently controversial. There is some evidence for activation of a *ras* gene and of *Blym*^{38,39}.

How do these genes interact in generating Burkitt's lymphoma? EBV is a uniquely potent immortalizing agent for human B cells. Three regions of the viral genome (LT-1, 2 and 3) are regularly transcribed in immortalized cells⁴⁰. The LT-1 region seems to be necessary for inducing the first activation step that is followed by a chain of events leading to indefinite proliferation in the normal B lymphocyte: an EBV substrain in which the LT-1 region has been deleted cannot activate and immortalize normal lymphocytes⁴¹. The protein product of LT-1 is a DNA-binding nuclear protein of relative molecular mass 81,000, designated EBNA-2 (refs 42-44). A second nuclear antigen, EBNA-1, which was identified earlier, is encoded by the LT-2 region⁴⁵. Repression of the lytic cycle, a prerequisite for the continuous proliferation of the virally transformed B lymphocyte, may require the interaction of EBNA-1, EBNA-2 and some B-cell specific cellular constituent⁴⁶.

Recent experiments of Gordon *et al.*⁴⁷ suggest that at least part of the immortalizing effect of EBV on the B lymphocyte results from the activation of an autocrine loop (for a recent review of autocrine activation see ref. 48). Both the EBV-transformed B-cell lines of normal origin (LCL) and the tumorigenic BL lines release B-cell growth factor (BCGF), but although LCLs are dependent on it, BL cells are not. Some BL lines are responsive, but others have lost their responsiveness⁴⁹. The course of events is strongly reminiscent of earlier studies on tumours in hormone-dependent tissues where a 'conditioned tumour', initially dependent on the hormonal imbalance that generated it, later becomes independent of the hormone and eventually unresponsive to it⁵⁰.

If EBV is responsible for the immortalization of BL cells, what does the *myc*/immunoglobulin translocation contribute to tumorigenesis? In the typical translocations, the *myc* oncogene breaks at its non-coding 5'-end, or at variable distances upstream of it⁵¹⁻⁶³. The coding exons of the gene are transposed to the chromosome containing the immunoglobulin heavy-chain genes, head-to-head with the immunoglobulin gene. In contrast, the variant translocations (those involving the immunoglobulin

light-chain genes) break the chromosome below the tail end of the gene⁶⁴⁻⁶⁶. In these cases, therefore, *c-myc* remains in its original location with the constant region of the κ or the λ light-chain attached to it, in a head-to-tail orientation. Despite these differences, all three translocations activate *myc* transcription constitutively. The importance of this activation and the intactness of the *myc* protein is stressed by the fact that the coding second and third exons of *c-myc* are always intact, despite the considerable variability of the translocation breakpoint in and around the gene. This variability and the equally large variation in the translocation breakpoints within the immunoglobulin genes also implies that no single enhancer or promoter can be responsible for *myc* activation in all or even most of the tumours. This may suggest that what is important is the active state of the chromatin, rather than single specific sites^{63,67}.

Early interpretations of the mechanism whereby the *c-myc*/immunoglobulin juxtaposition contributes to the tumorigenic process included claims of abnormally high transcription rates, abnormal transcript size, changed promoter use, mutations⁶⁸ and changed translational control. Each claim has been documented for some tumours, either in the BL or in the closely analogous mouse plasmacytoma (MPC) system, but none was found to apply to all or even most of the tumours. Perhaps there are several ways of activating *c-myc*, and no unifying mechanism exists⁶⁹. Alternatively, the crucial event may be more subtle than a relatively crude quantitative or qualitative change. The current concept is *dysregulation*. The idea is that the immunoglobulin-juxtaposed *myc* gene has become subject to *cis* control by the constitutively active immunoglobulin region and must therefore behave as if it were part of the immunoglobulin locus itself. The transcription of the normal *myc* gene is associated with competence for cell division^{70,71}; in terminally differentiating cells *c-myc* transcription ceases⁷². Mature plasma cells continue to produce immunoglobulins, however, and an immunoglobulin-juxtaposed *myc* may be similar. This would keep the cells in continuous division.

Although there is no direct evidence for this hypothesis, it is supported by comparative studies on the behaviour of the translocated *myc* gene and its normal allele in MPCs and BLs. It has been found repeatedly in both systems that the translocated gene is highly transcribed whereas its normal counterpart is switched off⁷³⁻⁷⁸. This is compelling evidence for the differential regulation of the two genes.

The BCGF-independence of the BL-cells may result from the locking of the immunoglobulin-juxtaposed *myc*-gene in a continuously active position. It may act as a 'pacemaker' from

Table 2 Expression of three oncogenes in three different tumours of the B-cell lineage, induced by Abelson virus, with or without mineral oil

Tumour	Inducing agent	Cell type	<i>v-abl</i> expression	<i>c-myb</i>	<i>c-myc</i>	Karyotype
ABLS	Abelson virus	Pre-B	+++	Normal	Normal	Normal diploid
ABPL	Abelson+oil	B?	—	+++ rearranged	Normal	No translocation
ABPC	Abelson+oil	Plasma cell	+++	Normal	+++ rearranged	12;15 or 6;15

(After the data of Mushinski and Potter *et al.*^{84,89-92}.)

within, prompting the continuous re-entry of the cells into the cycle, thereby obviating the need for exogenously supplied BCGF.

Pending further definition of the third oncogene and clarification of the important question of whether the DNA of tumours *in vivo* has the transforming activity that has been reported in the DNA of established BL lines, it may be noted that transforming *ras* genes with the characteristic point mutation have recently been isolated from certain experimental lymphomas^{9,79}. The mutant *ras* gene is believed to transform fibroblasts by inducing anchorage independence; but lymphocytes are inherently anchorage independent, so if mutant *ras* genes do play a part in the aetiology of BLs, it must be by some other mechanism. The attachment of the *ras* protein to the inner surface of the plasma membrane prompts speculations on membrane rearrangements that may interfere with the normal receptor organization and/or the transmission of growth and differentiation controlling signals.

In the natural history of African BL, EBV-transformation of the target cell is probably the first event⁸⁰. The environmental co-factor, perhaps chronic hyperendemic malaria⁸¹, may act by increasing the size of the target cell population. Because the EBV-infected B cell is longer-lived than its EBV-negative counterpart, it may undergo a larger number of divisions before being eliminated by terminal differentiation or by immunological mechanisms. The probability of all genetic aberrations increases with the number of cell divisions, including the risk of the *myc*/immunoglobulin juxtaposition. Heavy malarial infection may facilitate the development of the lymphoma in a dual capacity: polyclonal B-cell activation and T-cell immunosuppression. Table 1 is a schematic summary of our current view of the genesis of BL.

It is instructive to compare the course of events leading to BL with those culminating in the development of a mouse plasmacytoma. Molecular studies have confirmed our original suggestion⁸² that MPC and BL carry homologous translocations that involve the activation of the same oncogene by an immunoglobulin locus. The close homology of the translocations in these two tumours is remarkable, because their natural history is very different. MPC originates from mature plasma cells, whereas BL arises from a relatively immature B cell, producing the mainly surface rather than the secreted form of immunoglobulin⁸³. The human counterpart of mouse plasmacytoma is multiple myeloma, whereas the mouse equivalent of BL is B-cell lymphoma; these cells do not carry similar translocations. What then could account for their regular appearance in BL and MPC? A possible common denominator in MPC and BL is a long preneoplastic period in which the cells are chronically stimulated. Intraperitoneal granuloma induced by mineral oil is a prerequisite for MPC⁸⁴ whose development can be prevented by hydrocortisone which inhibits the formation of the granuloma. In BL, a functionally analogous chronic stimulation may be provided by the combination of EBV and chronic hyperendemic malaria.

No virus is known to be involved in the development of MPC and the BCGF-mediated autocrine loop may be missing. Note, however, that the mineral oil-induced MPCs are often not transplantable unless the syngeneic recipients carry an oil granuloma similar to that in the original host. This suggests that constitutive

activation of *myc* is not sufficient to produce a fully autonomous neoplasm in a single step. Therefore, it is of interest that several secondary changes have been repeatedly found in some MPCs. They include a 6;10 translocation that leads to the juxtaposition of *C-κ* and an unknown single-copy gene⁸⁵ or the activation of the *c-mos* oncogene by the insertion of an intracisternal A particle^{86,87} and a variety of other changes (see ref. 88 for review).

These secondary changes have been found in long-propagated lines of mouse plasmacytomas and may thus reflect changes in the tumour in culture. There is, however, one interesting example of an interaction occurring at the inception of the tumour. The induction of primary plasmacytomas can be facilitated greatly by the parallel administration of Abelson virus and mineral oil⁸⁴. The latency period is reduced and the frequency of plasmacytomas in the susceptible BALB/c strain increased. Abelson virus carries its own oncogene (*v-abl*). In the resulting tumours, the *v-abl* gene is transcribed at high levels, in parallel with the appearance of the typical 12;15 or the variant 6;15 translocation⁸⁹. The same treatment (combination of Abelson virus and mineral oil) also induced some plasmacytoid lymphosarcomas (ABPL), but they show a different pattern of oncogene activation. Mushinski *et al.*⁹⁰⁻⁹² have shown that these tumours carry rearranged activated *myb* genes, with a long terminal repeat (LTR) from a murine leukaemia virus inserted at the 5' end of the oncogene. The LTR seems to be derived from the Moloney helper virus. The *v-abl* oncogene is not usually expressed, nor is there any *myc* activation by translocation in these tumours.

These findings are summarized in Table 2. They are contrasted with the Abelson virus-induced pre-B-cell lymphomas in which *v-abl* is highly transcribed, but with no anomalies of *c-myb* or *c-myc* transcription. These experiments suggest that the transformation of cells in different stages of differentiation in the same lineage is brought about preferentially by different oncogenes. A similar situation may prevail in some of the lymphomas and the leukaemias of the human B-cell series. In Burkitt lymphoma and the closely related B-cell-derived acute lymphatic leukaemia, the *myc* oncogene is activated by its juxtaposition to one of the immunoglobulin loci. In contrast, other B-cell tumours may carry 14q markers generated by the attachment of other chromosome pieces to the IgH region of chromosome 14 (ref. 93). Recently, Croce's group has analysed the relatively common 11;14 and 14;18 translocations at the molecular level. They have identified two new potential oncogenes, designated *bcl-1* and *bcl-2*, derived from chromosomes 11 and 14, respectively⁹⁴.

Small-cell lung carcinoma

The recent work of Minna's group has interesting implications for the progression of small-cell or oat-cell carcinoma (SCLC) of the lung^{95,96}. SCLC exists in a typical relatively low malignant form and in a highly malignant variant form (*v*-SCLC). Three types of genetic change are found in this system. Most of all the tumours examined have a deletion in the short arm of chromosome 3. Shortest-region-overlap analysis has localized the consistent change to a small area (3p14). It may be significant that this region coincides with the breakpoint in two translocations associated with renal carcinoma (3;8 and 3;11)^{97,98}. It also corresponds to the approximate localization of the *raf* oncogene⁹⁹. The SCLC-associated deletion arises by a somatic

change and is not present in the normal cells of the patient. Although the deletion is visible only in one of the two homologous chromosomes, analysis by restriction-fragment-length polymorphism has revealed a deletion in the other chromosome 3 in the tumour cells of heterozygous patients (J. Minna, personal communication). This is consistent with Knudson's¹³ hypothesis that some tumours develop only after the loss of both alleles of a single gene.

Activation of a *ras* oncogene by point mutation is the second type of genetic change in SCLC¹⁰⁰⁻¹⁰². It is only found in a few cases, however, and its significance is therefore less clear.

The third genetic change is the most interesting in the sense that its phenotypic consequences are the easiest to interpret. In ~20% of SCLC tumours, or cell lines derived from them, *c-myc* or *N-myc* is amplified 20–80-fold, with substantially increased transcription. This change is regularly associated with the variant v-SCLC phenotype (ref. 95; J. Minna, personal communication). Such variants have increased cloning efficiency, shortened doubling times, decreased expression of differentiation markers and decreased adhesiveness compared with the typical tumour. In the patient, v-SCLC is more metastatic and has a poorer prognosis than the typical form. Occasionally, progression from the typical to the variant form can be observed in the same patient. The variant appears as a morphologically different subtype in the original tumour or in one or several metastases.

The amplification of *myc* in SCLC is often associated with the appearance of double minute chromosomes, or homogeneously staining regions that do not necessarily correspond to the original location of the amplified gene and whose locations may vary between tumours. In one case, cells from different metastatic sites in the same patient all had amplifications of the same *myc* gene, but in different arrangements.

These findings strongly suggest that the amplified genes can be very labile and that amplification of either *c-myc* or *N-myc* plays an important and perhaps decisive part in the transition of low malignant SCLC to the highly malignant variant phenotype. Moreover, the postulated double loss of one gene by deletion and the concurrent amplification of a *myc* gene strengthens the analogy with retinoblastoma in view of the recent demonstration of *N-myc* amplification in the latter system¹¹. It may also be noted that Minna's findings suggest, for the first time, a functional analogy between *c-myc* and *N-myc*.

The amplification of *myc* has also been found in other types of tumour, the promyelocytic leukaemia line HL60 being a particularly well-known example¹⁰³. This continuously growing cell line shows an extraordinarily high level of *c-myc* transcription, which, however, can be turned off very rapidly when the cell is induced to differentiate⁷². Amplification of *myc* has also been found in various solid human tumours¹⁰⁴ and in transplanted rat hepatomas¹⁰⁵. In neuroblastoma, *N-myc* amplification appears in stage III and IV tumours and may be related to tumour progression¹⁰⁶. Recently, we have found amplification of *c-myc* in a highly malignant plasma cell leukaemia, but not in the less malignant multiple myelomas¹⁰⁷.

It may be more than a curiosity that Bishop's group has detected amplification of *c-myc* in a 25-year old, originally polyoma-induced ascites tumour, SEWA¹⁰⁸. Double minute chromosomes were first discovered in this tumour¹⁰⁹, they carry amplified *c-myc* sequences.

These divergent examples raise a number of intriguing questions about the roles of the *myc* genes in the oncogenic process and in tumour progression; *v-myc* is the only known virally carried oncogene that can induce tumours in all three major tissues: epithelial, mesenchymal and haemopoietic. Similarly, *c-myc* activation by retroviral insertion in the chicken bursal lymphoma¹¹⁰ and by chromosomal translocation in MPC and BL represent early events in the history of these tumours. By contrast, amplification is usually a late event that occurs during the progression of established tumours.

Transcription of *myc* is switched on when the cell cycle is activated in both lymphocytes and fibroblasts^{70,71}. If *myc* plays a key part in this activation it is not surprising that the constitu-

tive activation of the gene by viral or cytogenetic accidents that bring it under the *cis* control of highly active regions may keep the cell continuously in cycle. This could happen in cells of many different types. It may be the first step in the chain of events that lead to tumour development, or a late step that prompts malignant progression of established tumours. This also implies that permanent activation of a *myc* gene ought to change certain non-tumorigenic cell lines that have the appropriate genetic constitution to tumorigenic variants. This has been demonstrated experimentally by Keath *et al.*¹¹¹. Rat fibroblast variants that became tumorigenic after transfection with appropriate *myc* constructs showed none of the usual morphological changes of transformed cells, and did not acquire high agarose clonability, but they did have a diminished requirement for serum. In these cells, the transfected gene was expressed at high levels whereas the normal *c-myc* allele was silent, just as the translocated *c-myc* gene is active and the normal allele silent in MPC and BL.

Progression never reaches an endpoint

The clonal evolution of tumour cell populations proceeds through the sequential selection of variant subpopulations from a common progenitor^{5,112-114}. New variants may arise at any time; some of them have a selective growth advantage over earlier forms. This process may be accelerated by the genetic instability of tumour cell populations, reflected by the high frequency of mitotic errors and variation of genetic markers. This instability may become more pronounced as the neoplasm evolves (see ref. 5 for review).

This process can be particularly clearly seen in the chromosomal evolution of chronic granulomatous leukaemia (GGL) carrying a characteristic chromosomal translocation giving rise to what is known as the Philadelphia (Ph¹) chromosome^{115,116}. During the early, chronic and relatively benign stage of the disease the precursor pool for the entire granulocyte, erythrocyte- and thrombocyte-forming bone marrow is taken over by a single clone of cells carrying the Ph¹ chromosome¹¹⁷. Occasionally, it may involve part of the bone marrow-derived (B) lymphocyte series as well. The Ph¹ (22q⁻) chromosome arises most frequently by a 9;22 translocation. In a variant minority, the exchange seems to involve chromosome 22 and virtually any other autosome.

When it was first discovered, the significance of the Ph¹ translocation was unknown. More recently however, as with the translocations characteristic of BL, it has become possible to look for an interpretation in terms of oncogene activation. In particular, the *c-abl* oncogene, which is located on the tip of chromosome 9, is regularly transposed to the deleted 22q⁻ chromosome in Ph¹-positive CGL^{118,119}. It is particularly significant that this happens not only in the typical 9;22 but also in the variant translocations¹²⁰ where the deleted distal arm of chromosome 22 moves to another autosome, not 9. The fact that *c-abl* moves also from 9 to 22 in these cases implies that three chromosomes are involved in the variant translocations. The consistency of the *abl* transposition greatly increases the probability that it is significant in tumorigenesis.

The molecular analysis of this transposition is still at an early stage. On chromosome 9, the breakpoint is immediately upstream of, or within, the *c-abl* oncogene¹²¹. On chromosome 22, the breakpoints are clustered within a limited 5–8-kilobase (kb) region, designated *bcr*¹²². Little is known about the level of expression, except the interesting fact that a new 8-kb *abl* RNA transcript can be found in Ph¹+CGL, whereas the 6- and 7-kb transcripts found in normal haematopoietic cells are absent¹²³. The functional significance of the transposed *abl*-carrying 22q⁻ chromosome, rather than the reciprocal 9q⁺ fragment, is supported indirectly by the finding that K562, a CGL-derived, originally Ph¹+ erythroleukaemia line, has amplified the chromosome carrying the transposed *abl* gene and its immediate surroundings, but not the reciprocally translocated *sis*-oncogene, located on chromosome 22 distally of the Ph¹ breakpoint¹²².

The appearance of the Ph¹ chromosome is the first clearly definable step in the development of Ph¹+CGL. Therefore, its initial action must occur during the early chronic phase of the disease, which has a median duration of 3 years. It usually precedes a second or acute phase, characterized by decreasing cell maturity and increasing blast proliferation. The transition from the chronic to the acute phase is often associated with further nonrandom chromosomal changes¹²⁴. The most frequent secondary events are the appearance of a second Ph¹ chromosome, or an additional chromosome 8, or an additional long arm of chromosome 17. Note that the trisomy of the *c-myc*-carrying chromosome 8 is the commonest trisomy in all human haemopoietic disorders, ranging from relatively benign myeloproliferative diseases to acute leukaemia¹¹⁵. This may be more than a coincidence, particularly if considered together with the fact that trisomy of chromosome 7 is the commonest chromosomal change in Rous sarcoma virus-induced rat tumours¹²⁵ and trisomy of chromosome 15 is the commonest and often single chromosomal anomaly in mouse leukaemia¹²⁶⁻¹²⁸. The *c-myc* gene has been localized to chromosome 7 in the rat¹²⁹ and to chromosome 15 in the mouse¹³⁰.

Immunology of host-tumour relationship

Widely polarized views prevail on the question of immune responses to tumours. At one extreme, they are believed to play a crucial part in protecting the host against neoplastic growth. At the other, they are regarded as virtually non-existent. The unfortunate tradition of considering this important problem in all-or-none terms often prevents a more discriminative analysis.

The probability of efficient immune rejection varies depending on the mode of tumour induction. It should be considered separately for tumours induced by DNA tumour viruses, those resulting from retrovirus insertion near a cellular oncogene, and tumours arising from oncogene activation by spontaneous or induced changes in cellular DNA. We shall consider each case in turn.

(1) Oncogenic DNA viruses carry their own transforming genes. This is why transformed cells of this category provide the best targets for host immune surveillance¹³¹. The evidence for such surveillance is indirect and can best be seen for man in the case of EBV. The immune mechanisms that normally suppress tumorigenesis by EBV are unknown. But it is clear that tumours develop only in special circumstances, for example in patients with relatively grave combined forms of congenital or acquired immunodeficiency. At least some of the fatal mononucleosis cases may belong in this category. The X-linked lymphoproliferative syndrome¹³² arises by the proliferation of EBV-carrying lymphoid cells in patients who lack virtually all T-cell activity, have no natural or interferon-activable killer cells and little or no immunoglobulin^{133,134}. Organ transplant recipients, who have a more restricted immune defect, also develop EBV-carrying lymphoproliferative lesions^{135,136}.

An interesting but unresolved question arising in connection with these tumours is whether they carry the chromosomal translocations characteristic of Burkitt lymphomas. Such translocations have now been found in EBV-carrying Burkitt lymphomas arising in patients with acquired immune deficiency syndrome (AIDS)¹³⁶. By contrast with Burkitt's lymphoma, which is typically a disease of childhood, Burkitt lymphomas associated with AIDS arise in adults between 20 and 40. The implication is, therefore, that EBV-positive, translocation carrying 'BL candidate' cells may arise at any time of life, but in normal immunocompetent individuals they will be eliminated by immune rejection. The immunosuppressive factor in the African childhood BLs may be chronic malaria⁸¹. Although T-cell mediated immune responses have been clearly demonstrated in the case of EBV-carrying BLs^{138,139}, there is no evidence for any host response against EBV negative BLs that carry the same translocations as the EBV positives, nor do they show the occasional spontaneous regressions or the ready curability by chemotherapy that is characteristic for the EBV-carrying and prognostically more favourable counterparts.

Note in this connection that the increase in malignant disease in congenitally or iatrogenically immunosuppressed patients is limited to a small and rather unusual group of tumours, such as lymphomas and other lymphoproliferative diseases, Kaposi's sarcoma, skin papilloma and carcinoma^{137,140}. It would not be surprising if most, if not all, of these tumours turned out to be DNA-virus related. EBV has already been found in most of the lymphoproliferative lesions in immunodeficiency^{133,135-138}. Cytomegalovirus is suspected in Kaposi's sarcoma¹⁴¹ and papilloma viruses may be partly or fully responsible for the skin tumours¹⁴².

(2) There is increasing evidence for cellular oncogene activation by retroviral promoter or enhancer insertion¹¹⁰ as a mechanism of tumorigenesis. Its relevance has been demonstrated for a variety of avian and mammalian tumours. Only a small part of the retroviral genome is required for the activation of the adjacent cellular oncogene, and that region contains only the regulatory LTR: it has no coding function. Although DNA encoding some of the viral proteins (*gag* and *env*) may also be present, the proteins are not necessary and may even be disadvantageous for oncogene activation because they provide potential targets for the immunological response of the host. Given that the protein-coding regions of the virus may be lost while the transformed phenotype of the cell is maintained, it is not surprising that immune rejection of tumour induced by RNA tumour viruses is unpredictable.

(3) Activation of cellular oncogenes by nonviral mechanisms such as point mutations, DNA rearrangements, gene amplification and chromosomal translocations, is probably the basis for most of the spontaneous tumours. The transforming oncogene products are either normal or only slightly modified cellular proteins. It is difficult to see how they could provide a rejection target for the immune system. It is at least possible that a mutated ras protein with a changed amino acid at position 12 or 61 could be antigenic, but this has not yet been demonstrated.

Thus, it seems that specific immune recognition and rejection of antigenically foreign tumour cells is largely restricted to virally induced tumours, and in particular to those caused by DNA viruses. There is increasing evidence, however, that rejection by specifically sensitized T cells is only one of several effector mechanisms that can counteract neoplastic target cells. Another example is provided by natural cytotoxicity that can play a significant role in protecting the host against the metastatic growth of disseminated tumour cells^{143,144}. Natural killer (NK) cells can kill sensitive tumours without having been immunized against specific antigens on the target cell surface. Their action is not restricted to virally or chemically induced neoplasms, although different tumours differ in their NK sensitivity. NK cells may be scavengers that can recognize and eliminate 'displaced' cells that have wandered outside their natural homing boundaries. Cells in which the expression of 'self' markers encoded in the MHC is reduced are particularly sensitive targets²⁶. It is intriguing that diminished expression of MHC class I antigens can enable tumour cells to avoid rejection by specifically sensitized MHC-restricted cytotoxic T cells, but increases the probability of their elimination by NK cells. Conversely, there is evidence that an increased expression of MHC antigens in mice may increase metastasis^{27-29,145}. This, then, is a case in which a quantitative change in a universally occurring cell surface marker can either enhance or inhibit tumour progression.

Future of molecular oncology

In his classical paper on tumour progression⁴, Foulds emphasized that most tumours develop by stepwise changes in many independent characteristics. The independent assortment of these characteristics leads to the many individual variations in the biological properties of tumours. Foulds stressed the need for a factorial analysis of the complex tumour phenotype. The advent of modern molecular biology has made such an analysis possible. We are at the threshold of a previously unknown world where oncogenes, antioncogenes and other cellular genes inter-

act within complex regulatory circuits. Thanks to the recombinational accidents of the retroviral lifestyle, we now have access to the dominantly acting oncogenes as an unexpected gift from viral oncology. So far, we have learned much at the level of the DNA sequence, something of transcriptional regulation, and a little of the mechanisms whereby the oncogene products can change cellular behaviour. At least two other worlds of genes, so far less conspicuous, remain unexplored. The suppression of tumorigenicity in hybrids between normal and malignant cells and the regular loss of both alleles of a single gene in the genesis of such tumours as retinoblastoma or Wilms' tumour suggest the existence of other, probably quite heterogeneous gene families, tentatively designated anti-oncogenes or tumour suppressor genes that may counteract the expression of the tumorigenic phenotype. There may be also special safeguards to prevent the inadvertent activation of particularly dangerous oncogenes, as illustrated by the example of *c-mos*¹⁴⁶, which is

never transcribed in normal cells and even a low level of transcription seems to be sufficient for neoplastic transformation. A 5' flanking sequence prevents the downstream activation of the gene in the mouse. The human *c-mos* gene shows an even more drastic change, a gap in the middle that may prevent activation altogether. These and other existing hints indicate that evolution may have fixed a whole variety of protective mechanisms that may *cis-* or *trans-*regulate the expression of activated oncogenes. A third and probably even more widely diversified world of genes is not directly involved in the initiation of neoplastic transformation or in the protection against it, but can nevertheless affect the phenotypic properties of established tumours in a prognostically important way. Proteolytic mechanisms that contribute to invasiveness, changes in 'homing markers' that can influence the neoplastic colonization of distant organs, and changes in the ability of tumour cells to induce or to succumb to an immune response fall into this category.

1. Farber, E., & Cameron, R. *Adv. Cancer Res.* **31**, 125-226 (1980).
2. Knudson, A. *Adv. Cancer Res.* **17**, 317 (1973).
3. Foulds, L. *Cancer Res.* **14**, 327-339 (1954).
4. Foulds, L. *J. Chron. Dis.* **8**, 2-37 (1958).
5. Nowell, P. C. *Science* **194**, 23-28 (1976).
6. Temin, H. *J. Cell Physiol. Suppl.* **3**, 1-11 (1984).
7. Rous, P. & Beard, J. W. *J. exp. Med.* **69**, 399-424 (1939).
8. Klein, G. (ed.) *Advances in Viral Oncology* Vol. 1 (Raven, New York, 1982).
9. Barbacid, M. *3rd Duran Reynolds Int. Symp., Barcelona* (in the press).
10. Cavenee, W. K. *et al. Nature* **305**, 779-784 (1983).
11. Murphree, A. L. & Benedict, W. F. *Science* **223**, 1028-1033 (1984).
12. Benedict, W. F. *et al. Science* **219**, 973-975 (1983).
13. Knudson, A. G. *Proc. natn. Acad. Sci. U.S.A.* **68**, 820-823 (1971).
14. Knudson, A. G. & Strong, L. C. *Am. J. hum. Genet.* **24**, 514 (1972).
15. Comings, E. E. *Proc. natn. Acad. Sci. U.S.A.* **70**, 3324-3328 (1973).
16. Robertson, M. *Nature* **309**, 512-513 (1984).
17. Harris, H. *et al. Nature* **223**, 363-368 (1969).
18. Klein, G. *et al. J. Cell. Sci.* **8**, 659-672 (1981).
19. Wiener, F. *et al. J. Cell. Sci.* **12**, 253-261 (1973).
20. Stanbridge, E. J. *Nature* **260**, 17-20 (1976).
21. Jonasson, J. *et al. J. Cell Sci.* **24**, 217 (1977).
22. Spira, J. *et al. Int. J. Cancer* **28**, 785-798 (1981).
23. Evans, E. P. *et al. J. Cell Sci.* **56**, 113-130 (1982).
24. Klinger, H. P. & Shows, T. B. *J. natn. Cancer Inst.* **71**, 559-569 (1983).
25. Noda, M. *et al. Proc. natn. Acad. Sci. U.S.A.* **80**, 5602-5606 (1983).
26. Taniguchi, K., Kärre, K. & Klein, G. *Int. J. Cancer* (in the press).
27. De-Baetselier, P. *et al. Nature* **288**, 199 (1980).
28. Katzav, S. *et al. J. natn. Cancer Inst.* **71**, 317 (1983).
29. Sanderson, A. R. & Beverley, P. E. *Immun. Today* **4**, 2111 (1983).
30. Nicolson, G. L. *Biochim. biophys. Acta* **695**, 113-176 (1982).
31. Danö, K. *et al. Adv. Cancer Res.* **92**, 140-239 (1985).
32. Dalianis, T. *et al. Immunogenetics* **9**, 465-475 (1979).
33. Fenyö, E. M. & Klein, G. *Nature* **260**, 355-357 (1976).
34. Geser, A. *et al. Int. J. Cancer* **29**, 297-400 (1982).
35. Klein, G. *Cold Spring Harb. Symp. quant. Biol.* **39**, 783-790 (1975).
36. Klein, G. & Klein, E. *Carcinogen* **5**, 429-435 (1984).
37. Klein, G. *Cell* **32**, 311-315 (1983).
38. Murray, M. J. *et al. Cell* **33**, 749-757 (1983).
39. Diamond, A. *Nature* **305**, 112-116 (1983).
40. Kieff, E. *et al. Adv. Viral Oncol.* **3**, 133-182 (1983).
41. Menezes, J. *et al. Exp. Cell Res.* **92**, 478-484 (1975).
42. Rymo, L., & Klein, G. *Proc. natn. Acad. Sci. U.S.A.* (in the press).
43. Dillner, J., Kallin, B. *et al. Int. J. Cancer* **35**, 359-366 (1985).
44. Hennessy, K. & Kieff, E. *Proc. natn. Acad. Sci. U.S.A.* **80**, 5665-5669 (1983).
45. Summers, W. *et al. Proc. natn. Acad. Sci. U.S.A.* **79**, 5688-5692 (1982).
46. Klein, G. & Klein, E. *Prog. Med. Virol.* **30**, 87-106 (1984).
47. Gordon, J. *et al. Nature* **310**, 145-147 (1984).
48. Sporn, M. & Roberts, A. B. *Nature* **313**, 745-747 (1985).
49. Gordon, J. *et al. Int. J. Cancer* **35**, 251-256 (1985).
50. Furth, J. *Cancer Res.* **13**, 477-492 (1953).
51. Leder, P. *et al. Science* **222**, 765-771 (1983).
52. Shen-Ong, G-L. *et al. Cell* **31**, 443-452 (1982).
53. Adams, J. M. *et al. Proc. natn. Acad. Sci. U.S.A.* **79**, 6966-6970 (1982).
54. Adams, J. M. *et al. Proc. natn. Acad. Sci. U.S.A.* **80**, 1982-1986 (1983).
55. Cory, S. *et al. EMBO J.* **2**, 213-216 (1983).
56. Cory, S. *et al. Science* **219**, 963-967 (1983).
57. Dalla-Favera, R. *et al. Science* **219**, 963-967 (1983).
58. Erikson, J. *et al. Proc. natn. Acad. Sci. U.S.A.* **80**, 820-824 (1983).
59. Taub, R. *et al. Proc. natn. Acad. Sci. U.S.A.* **79**, 7837-7841 (1982).
60. Calame, K. *et al. Proc. natn. Acad. Sci. U.S.A.* **79**, 6994-6998 (1982).
61. Harris, L. J. *et al. Proc. natn. Acad. Sci. U.S.A.* **79**, 4175-4179 (1982).
62. Stanton, L. W. *et al. Nature* **303**, 401-406 (1983).
63. Perry, R. P. *Cell* **33**, 647-649 (1983).
64. Croce, C. M. *et al. Proc. natn. Acad. Sci. U.S.A.* **80**, 6922-6926 (1983).
65. Erikson, J. *et al. Proc. natn. Acad. Sci. U.S.A.* **80**, 7581-7585 (1983).
66. Emanuel, B. S. *et al. Proc. natn. Acad. Sci. U.S.A.* **81**, 2444-2446 (1984).
67. Weisbrod, S. *Nature* **297**, 289-295 (1982).
68. Rabbitts, T. H. *et al. Nature* **309**, 592-597 (1984).
69. Robertson, M. *Nature* **309**, 585-587 (1984).
70. Kelly, K. *et al. Cell* **35**, 603-610 (1983).
71. Campisi, J. *et al. Cell* **36**, 241-247 (1984).
72. Reitsma, P. H. *et al. Nature* **306**, 492-494 (1983).
73. Croce, C. M. & Klein, G. *Scient. Am.* **252**(3), 54-60 (1985).
74. Adams, J. M. *et al. Proc. natn. Acad. Sci. U.S.A.* **80**, 1982-1986 (1983).
75. Erikson, J. *et al. Proc. natn. Acad. Sci. U.S.A.* **80**, 820-824 (1983).
76. Harris, L. J. *et al. Proc. natn. Acad. Sci. U.S.A.* **79**, 4175-4179 (1982).
77. Stanton, L. W. *et al. Nature* **303**, 401-406 (1983).
78. Leder, P. *et al. Science* **222**, 765-771 (1983).
79. Guerrero, I. *3rd Duran Reynolds Int. Symp., Barcelona* (in the press).
80. Klein, G. *Proc. natn. Acad. Sci. U.S.A.* **76**, 2442-2446 (1979).
81. Burkitt, D. P. *J. natn. Cancer Inst.* **42**, 19-28 (1969).
82. Klein, G. *Nature* **294**, 313-318 (1981).
83. Nilsson, K. & Klein, G. *Adv. Cancer Res.* **37**, 319-380 (1982).
84. Potter, M. *et al. Adv. Viral Oncol.* **4**, 139-162 (1984).
85. Perlmutter, R. M. *et al. Nature* **307**, 473-476 (1984).
86. Rechavi, G. *et al. Nature* **300**, 607-611 (1982).
87. Canaani, E. *et al. Proc. Natn. Acad. Sci. U.S.A.* **80**, 7118-7122 (1983).
88. Klein, G. in *Genetic Rearrangements in Leukaemia and Lymphoma* (eds Goldman, J. M. & Harnden, D. G.) (Churchill Livingstone, London, in the press).
89. Ohno, S. *et al. J. exp. Med.* **159**, 1762-1777 (1984).
90. Mushinski, J. F. *et al. Science* **220**, 795-798 (1983).
91. Shen-Ong, G. L. C. *et al. Curr. Top. Microbiol. Immun.* **113**, 41-46 (1984).
92. Lavu, S. *et al. Curr. Top. Microbiol. Immun.* **113**, 37-40 (1984).
93. Rowley, J. *Cancer Genet. Cytogenet.* **1**, 263-271 (1981).
94. Tsujimoto, Y. *et al. Science* **224**, 1403-1406 (1984).
95. Little, C. D. *et al. Nature* **306**, 194-196 (1983).
96. Whang-Peng, J. *et al. Science* **215**, 181-182 (1982).
97. Cohen, A. J. *New Engl. J. Med.* **301**, 592-595 (1979).
98. Pathak, S. *et al. Science* **217**, 939-941 (1982).
99. Bonner, T. *et al. Science* **223**, 71-74 (1984).
100. Santos, E. *et al. Science* **223**, 661-664 (1984).
101. Nakano, H. *et al. Proc. natn. Acad. Sci. U.S.A.* **81**, 71-75 (1984).
102. Capon, J. J. *et al. Nature* **304**, 507-510 (1982).
103. Collins, S. & Groudine, M. *Nature* **298**, 679-681 (1982).
104. Alitalo, K. *et al. Proc. natn. Acad. Sci. U.S.A.* **80**, 1707-1711 (1983).
105. Hayashi, K. *et al. Gann* **75**, 475-478 (1984).
106. Brodeur, G. M. *et al. Science* **224**, 1121-1124 (1984).
107. Sümegi, J. *et al.* (in preparation).
108. Schwab, M. *et al. Nature* (in the press).
109. Levan, G. *et al. Hereditas* **86**, 75-90 (1977).
110. Hayward, W. S. *et al. Nature* **290**, 475-480 (1981).
111. Keath, E. J. *et al. Cell* **39**, 339-348 (1984).
112. Makino, S. *Ann. N. Y. Acad. Sci.* **64**, 818-822 (1956).
113. Levan, A. & Bieseke, J. *Ann. N. Y. Acad. Sci.* **72**, 1022-1026 (1968).
114. Hauschka, T. S. *Cancer Res.* **21**, 957-961 (1961).
115. Rowley, J. *Proc. natn. Acad. Sci. U.S.A.* **72**, 152-155 (1975).
116. Levan, G. & Mitelman, F. *Hereditas* **84**, 1-14 (1976).
117. Jacobsen, R. J. *et al. New Engl. J. Med.* **310**, 1513-1517 (1984).
118. Heisterkamp, N. *et al. Nature* **299**, 747-750 (1982).
119. DeKlein, A. *et al. Nature* **300**, 765-767 (1982).
120. Bertram, C. R. *et al. Nature* **306**, 277-280 (1983).
121. Heisterkamp, N. *et al. Nature* **306**, 239-242 (1983).
122. Groffen, J. *et al. Cell* **36**, 93-99 (1984).
123. Canaani, E. *et al. Lancet* **ii**, 593-595 (1984).
124. De Grouchy, J. & Turleau, C. in *Chromosomes and Cancer* (ed. German, J.) 287-298 (Wiley, New York, 1974).
125. Levan, G. & Mitelman, F. *Hereditas* **79**, 156 (1970).
126. Wiener, F. *et al. J. natn. Cancer Inst.* **61**, 227-238 (1978).
127. Wiener, F. *et al. Int. J. Cancer* **22**, 447-453 (1978).
128. Klein, G. *Nature* **294**, 313-318 (1981).
129. Sümegi, J. *et al. Nature* **306**, 497-498 (1983).
130. Cory, S. *et al. EMBO J.* **2**, 697-703 (1983).
131. Klein, G. & Klein, E. *Proc. natn. Acad. Sci. U.S.A.* **74**, 2121-2126 (1977).
132. Purtilo, D. T. & Klein, G. *Cancer Res.* **41**, 4209 (1981).
133. Saemundsen, A. K. *et al. Cancer Res.* **41**, 4237-4242 (1981).
134. Masucci, N. G. *et al. Cancer Res.* **41**, 4284-4291 (1981).
135. Hanto, D. W. *et al. Cancer Res.* **41**, 4253-4261 (1981).
136. Saemundsen, A. K. *et al. Lancet* **ii**, 158 (1982).
137. Ziegler, J. *Adv. Viral Oncol.* **5**, 239-255 (1985).
138. Rickinson, A. B. & Moss, D. T. *Adv. Viral Oncol.* **3**, 213-238 (1983).
139. Szigeti, R. *et al. Proc. natn. Acad. Sci. U.S.A.* **81**, 4178-4182 (1984).
140. Penn, I. *Adv. Cancer Res.* **28**, 32-61 (1978).
141. Beth, E. & Girado, G. *Int. J. Cancer* **26**, 23-29 (1980).
142. zur Hausen, H. *3rd Duran Reynolds Int. Symp., Barcelona* (in the press).
143. Hanna, N. & Burton, R. C. *J. Immun.* **127**, 1753-1758 (1981).
144. Gorelik, E. & Bere, W. W. *Int. J. Cancer* **33**, 87-94 (1984).
145. Dalianis, T. *et al. Immunogenetics* **12**, 371-380 (1981).
146. Blair, D. G. *et al. in Cancer Cells* (ed. van de Woude, G. F.) 281-289 (Cold Spring Harbor Laboratory, New York, 1984).

Large-scale regional units in the depleted upper mantle revealed by an isotope study of the South-West Indian Ridge

Bruno Hamelin & Claude J. Allegre

Laboratoire de Géochimie et Cosmochimie, Institut de Physique du Globe and Département des Sciences de la Terre, 4 Place Jussieu, 75230 Paris Cedex 05, France

Isotope analyses of ocean island basalts have demonstrated the existence of regional geochemical provinces, the Indian Ocean being distinguishable from the North Atlantic and Pacific oceans. Equivalent analyses of basalts from the South-West Indian Ridge now indicate that the upper mantle under the Indian Ocean also has a distinct isotope composition. Thus, the upper mantle itself must be composed of several discrete provinces which may relate to the convection pattern.

INTERPRETATION of isotope variations observed in basalts from mid-ocean ridges (MORB) is used currently to infer the structure of the upper mantle and the processes acting at ridge crests¹⁻³. Studies of the Mid-Atlantic Ridge (MAR)⁴⁻⁸, the East Pacific Rise (EPR)⁹ and Juan de Fuca and Gorda ridges¹⁰ have shown that the isotope results for MORB from these three ridges define common linear correlations in the Pb/Pb, Pb/Sr and Sr/Nd diagrams. These 'MORB' alignments are often interpreted as evidence of mixing between two types of mantle sources under the ridges. The first source is convecting asthenospheric material, corresponding to the 'depleted values', less radiogenic for Pb and Sr and more radiogenic for Nd, whereas the second is composed of blobs thought to be the source material of ocean island basalts and is characterized by more radiogenic Pb and Sr and less radiogenic Nd compositions. This model is based partly on the fact that isotope results for islands of the North Atlantic and East Pacific oceans extend the MORB alignments for these oceans in all the diagrams^{5,11-13}.

The study of the Indian Ocean allows an interesting test of this model, because it has been demonstrated^{11,14,15} that Indian Ocean islands have isotope compositions which are not on the extension of the Atlantic and Pacific MORB alignments in the Pb/Sr diagram. Dupré and Allegre¹⁵ have given preliminary results for MORB samples of the Carlsberg Ridge which suggest that such a regional characteristic of the Indian Ocean is also found in ridge products; the present study of the South-West Indian Ridge (SWIR) further documents this.

The SWIR is interesting because two hotspots, the isotope compositions of which have already been determined, are very close to it: Bouvet^{11,16}, on the south-west extremity, and Marion-Prince Edward (S. R. Hart and A. J. Erlank, manuscript in preparation), on the centre of the ridge. Le Roex *et al.*¹⁷ have measured Nd and Sr isotope compositions of samples from the south-west part of the SWIR, whereas we report here the Pb, Sr and Nd isotope results for the north-east part.

Samples

The samples from the SWIR analysed here were collected during the MD34 cruise of the RV *Marion Dufresnes* in February 1983. Eight dredging locations were spaced regularly from the Atlantis fracture zone (57°E) (at 13° longitude from the Rodriguez triple junction) to the Prince Edward fracture zone (35°E). The ridge rises continuously from depths of ~4,000 m at the triple junction up to the swelling where the islands of Marion and Prince Edward emerge near the ridge. This bathymetric swelling also corresponds to the convergence of the Madagascar plateau to the north and the Crozet plateau to the south-west (Crozet Island itself being 770 km from the ridge at this point).

The dredges were all performed in the axial valley, clearly identifiable by its magnetic signature. The centre of each ridge section delimited by major fracture zones was chosen for samp-

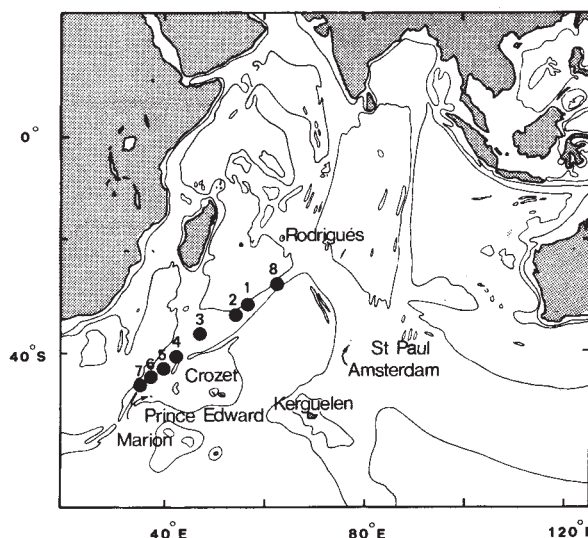


Fig. 1 Bathymetric chart of the Indian Ocean showing the location of the height dredges of the MD34 *Marion Dufresnes* cruise.

ling (Fig. 1). The dredge D8 (62°E) yielded only serpentinites, but in all other cases variable amounts of basaltic glasses were obtained.

Two samples from the MAR have been added to this set of samples, to assess the differences between the two oceans. Both samples are from the Kane fracture zone region, which has given some of the least radiogenic Pb and Sr isotope results^{9,18}. One of these samples (AII 96 18-1) had already been analysed for Sr and Nd by Machado *et al.*¹⁸.

Analytical procedures

Between 100 mg and 700 mg of glass was chosen, using a binocular microscope to eliminate any pieces of basalt with visible signs of alteration, encrusted sediments or plagioclase phenocrysts. Pb, Sr and Nd separations were performed using the standard procedures of Manhès *et al.*¹⁹, Birck and Allegre²⁰ and Richard *et al.*²¹. Powders obtained by crushing millimetre-sized pieces of glass in a boron carbide mortar were leached in 6 M HCl before dissolution, to remove marine Sr contamination.

Pb, Sr and Nd isotope compositions have been determined sometimes on the same powder sample. Pb was first separated in 0.5 M HBr solution after dissolving the powder in HF and HBr; fluoride residues were next eliminated by dissolving in HClO₄ and evaporation before Sr and Nd separations. Blank levels and standard calibrations are shown in Table 1.

Table 1 Pb, Sr and Nd isotope data for SWIR glasses

Sample	Location	Depth (km)	$^{206}\text{Pb}/^{204}\text{Pb}$	$^{207}\text{Pb}/^{204}\text{Pb}$	$^{208}\text{Pb}/^{204}\text{Pb}$	$^{87}\text{Sr}/^{86}\text{Sr}$	$^{143}\text{Nd}/^{144}\text{Nd}$
D1	31°41.6' S 57°50.5' E	4,050	17.554 ± 12 17.497 ± 18	15.433 ± 15 15.399 ± 16	37.289 ± 46 37.181 ± 51	0.70275 ± 2 0.70276 ± 2*	0.513081 ± 20
D2	33°45.3' S 56°16.4' E	3,800	18.114 ± 18	15.505 ± 26	37.839 ± 65	0.70269 ± 1* 0.70269 ± 2	0.512981 ± 22 0.513001 ± 28
D3	37°42.6' S 49°52.9' E	3,260	18.137 ± 11	15.491 ± 15	37.894 ± 48	0.70269 ± 3	
D4	40°59.0' S 43°42.0' E	3,700	17.767 ± 12 17.761 ± 15	15.467 ± 16 15.468 ± 18	37.641 ± 49 37.624 ± 60	0.70293 ± 2 0.70299 ± 3	0.513068 ± 34
D5	43°53.6' S 40°39.2' E	2,550	16.959 ± 17 16.927 ± 13	15.490 ± 20 15.504 ± 16	37.312 ± 65 37.320 ± 48	0.70475 ± 3* 0.70470 ± 3	0.512448 ± 60
D6	44°10.8' S 38°47.7' E	2,190	18.373 ± 11	15.509 ± 14	38.096 ± 46	0.70280 ± 3* 0.70281 ± 2	0.513071 ± 54
D7	44°48.4' S 36°18.1' E	1,500	18.237 ± 13	15.499 ± 15	37.976 ± 47	0.70280 ± 3	0.513041 ± 26
D8	28°44.4' S 62°02.0' E	5,250	18.362 ± 17 18.330 ± 13	15.585 ± 17 15.612 ± 16	38.050 ± 55 38.107 ± 53	0.70781 ± 2 0.70699 ± 5	
Residue leaching			18.327 18.302	15.561 15.597	37.835 38.195		
CH77	23°42' N	4,200	18.141 ± 13	15.456 ± 16	37.514 ± 49	0.70230 ± 3*	0.513203 ± 36
DR04 106	45°26' W						
AI1 96	23°31.5' N 44°49.1' W		18.015 ± 15	15.463 ± 18	37.438 ± 51	0.70233 ± 3*†	

Pb isotope results are calibrated against SRM 981 measurements, with a discrimination factor of 0.1% per mass unit. Errors are 1σ uncertainties taking into account the uncertainty of this discrimination correction⁹. Pb concentrations have been determined in most cases using a ^{203}Pb spike, and range from 290 to 700 parts per 10^9 (except for the serpentinite D8, 50 parts per 10^9). Sr isotopes are measured with a CAMECA double collector. The results are calibrated against the NBS 987 standard, whose measured value is 0.710254 ± 2 (23 measurements). The results are then corrected to the 0.71015 value previously measured with a single collector spectrometer. Errors are 2σ on the mean uncertainties. Duplicates are separate determinations on different glasses from the same dredge. Nd isotopes are measured with double filament technique with H_3PO_4 loading. The results are calibrated against a Johnson Matthey 321 standard, whose measured value is 0.511959 ± 8 (11 runs). Pb blanks are controlled routinely and are always < 0.7 ng (the blank correction is then much lower than uncertainties within the run). Sr blanks are < 1 ng; Nd blanks are < 0.1 ng.

* Pb/Sr/Nd analysis on the same dissolution.

† Value from Machado *et al.*¹⁸: 0.70233 ± 3 .

Results

Pb/Pb diagrams. As already found for other ridges, the Pb isotope results are correlated positively in both $^{207}\text{Pb}/^{204}\text{Pb}$ against $^{206}\text{Pb}/^{204}\text{Pb}$ and $^{208}\text{Pb}/^{204}\text{Pb}$ against $^{206}\text{Pb}/^{204}\text{Pb}$ diagrams (Fig. 2). The dredge D5 is an exception and will be discussed later.

The most salient feature of these results is that the least radiogenic extremity of the Pb/Pb correlations of the SWIR is clearly different from that obtained for the Pacific and Atlantic oceans; these values are less radiogenic for $^{206}\text{Pb}/^{204}\text{Pb}$ (17.5) but comparable for $^{207}\text{Pb}/^{204}\text{Pb}$ (15.43) and $^{208}\text{Pb}/^{204}\text{Pb}$ (37.3). This can be verified in Fig. 2 where the isotope domains obtained for other oceans^{1,7-9} have been plotted for comparison. The two Atlantic samples analysed during the present study (Table 1) confirm that this distinction is indeed real and cannot result from analytical bias.

At the other extremity of the SWIR Pb/Pb correlations, the values obtained are equivalent to the results for common MORB in the Atlantic and Pacific oceans. As a consequence, the Pb/Pb slopes calculated in both diagrams are lower for the SWIR than for the MAR and EPR. Note that these results are consistent with those of Dupré and Allègre¹⁵ on the Carlsberg Ridge, which plot exactly on the same correlation in the $^{208}\text{Pb}/^{204}\text{Pb}$ against $^{206}\text{Pb}/^{204}\text{Pb}$ diagram.

The dredge D5 sample displays a very different lead isotope composition. It has the smallest radiogenic $^{206}\text{Pb}/^{204}\text{Pb}$ (16.96) so far measured for an oceanic sample, and its $^{207}\text{Pb}/^{204}\text{Pb}$ (15.49) and $^{208}\text{Pb}/^{204}\text{Pb}$ (37.31) are distinctly above the MORB Pb/Pb correlations. Such values are exceptional for zero age mantle samples. Samples from islands such as Kerguelen¹⁴,

Tristan da Cunha and Gough¹¹ or from aseismic ridges such as Walvis²² and Ninety-East¹⁵ also have $^{207}\text{Pb}/^{204}\text{Pb}$ and $^{208}\text{Pb}/^{204}\text{Pb}$ ratios above the MORB correlation, but their $^{206}\text{Pb}/^{204}\text{Pb}$ are more radiogenic than the D5 value.

Finally, the peridotite D8 (not plotted) has a $^{207}\text{Pb}/^{204}\text{Pb}$ (15.58) slightly higher than the basalts of similar $^{206}\text{Pb}/^{204}\text{Pb}$ (18.36).

Pb/Sr diagram. The range of $^{87}\text{Sr}/^{86}\text{Sr}$ variation is from 0.7027 to 0.7030, again with the exception of D5 (0.7047; Fig. 3). These Sr values are higher than those obtained on the EPR and MAR for comparable $^{206}\text{Pb}/^{204}\text{Pb}$, and again this difference is confirmed by the two new results on Atlantic samples. The data for the SWIR are then above the Pb/Sr correlation discovered by Dupré and Allègre⁷ and Cohen *et al.*⁸ for the Atlantic and confirmed by Hamelin *et al.*⁹ for the Pacific. Again, this feature is also found for the Carlsberg samples^{8,15,23} and seems to be a regional characteristic of Indian MORB.

The unusual dredge D5 is far removed from the usual depleted mantle domain, with a $^{87}\text{Sr}/^{86}\text{Sr}$ of 0.70472. Again, this value is similar to those obtained on Kerguelen Island¹⁴ or the Walvis Ridge²².

The peridotite D8 gave a $^{87}\text{Sr}/^{86}\text{Sr}$ of 0.7071. Such high values, described in ophiolite peridotites^{24,25}, have been interpreted as evidence of interaction with sea water in oceanic layer 3.

Nd/Sr diagram. As for Sr, the range of Nd variation is restricted (0.51307–0.51291). Note that these results display less variation than those near the Bouvet hotspot of Le Roex *et al.*¹⁷. The data are in the usual Sr/Nd correlation for MORB. The ϵ_{Nd} values (5.7–8.8) are lower than those of the most depleted MORB of the Atlantic^{8,18} and Pacific²⁶, and comparable to intermediate

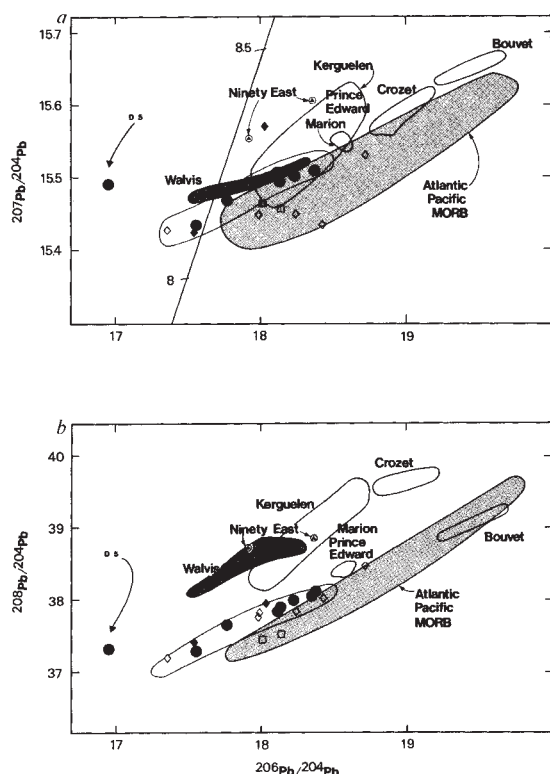


Fig. 2 *a*, Pb/Pb (α, β) diagram; *b*, Pb/Pb (α, γ) diagram. ●, SWIR (this study); ◆, SWIR data¹⁵; ◇, Carlsberg ridge data^{15,23}. □, Kane fracture zone (this study). References of the islands and Atlantic and Pacific MORB are given in the text, except for the Crozet field (W. M. White and B. Dupré, unpublished data). The geochron is calculated for an age of the Earth $T_0 = 4.55 \times 10^9$ yr. Two values of μ ($^{238}\text{U}/^{204}\text{Pb}$) are given as reference.

values such as those of the Reykjanes Ridge near Iceland²⁷. Their Sr/Nd compositions would then seem to be somewhat more 'enriched' than Atlantic MORB whereas the Pb/Pb results are more depleted.

The D5 dredge is again exceptional, with a $^{143}\text{Nd}/^{144}\text{Nd}$ of 0.512448 ($\epsilon_{\text{Nd}} = -3.3$), comparable with those of Kerguelen²⁸ or Walvis Ridge²² samples.

Geographical distribution of the isotope variations. The least radiogenic Pb and Sr values of the SWIR are found on the eastern part of the ridge, which corresponds to the deepest portion and to the triple junction zone. Dupré and Allègre¹⁵ reported a similar nonradiogenic result for a sample dredged at the triple junction.

At the opposite extreme, the more radiogenic values (D6 and D7) correspond to the shallowest part of the ridge, near the Prince Edward and Marion islands, and the isotope values approach those obtained by Hart and Erlank (manuscript in preparation) for basalts from these islands.

Discussion

Present-day source mixing under the SWIR. Isotope variations along ridges are thought to result from mixing between asthenospheric material and blobs of more radiogenic material. This has been well documented near Iceland^{4,5}, the Azores^{6,7,9} and Bouvet¹⁷ Island for the MAR and for the Galapagos spreading centre³⁰ near the Galapagos archipelago. In all these areas, hotspot influence on the ridges is well known from geochemical and bathymetric evidence²⁹.

A more complete sampling of the SWIR will be needed to investigate fully the isotope gradient away from Marion and Prince Edward islands, but it is clear that dredges located on the bathymetric swell of the ridge (D6, D7) display Pb and Sr isotope results analogous to those of the islands (S. R. Hart and

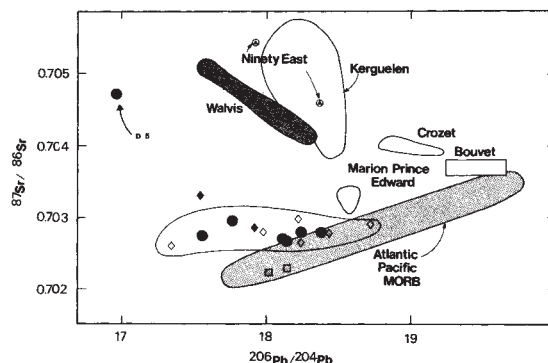


Fig. 3 Pb/Sr diagram. Symbols as described in Fig. 2 legend.

A. J. Erlank, in preparation) and that these results are thus in agreement with the model of present-day mixing for ridges.

Note that Pb seems to be the most sensitive tracer for this mixing process, because the Nd and Sr ranges of isotope variation remain smaller than for Pb. This suggests a larger difference in isotope composition between the asthenosphere and blob material for Pb than for Nd and Sr.

The question of the identification of the exact isotope composition of the enriched end-member cannot be solved only from isotope arguments, because the results measured on the islands are themselves representative of mixtures between blob and asthenospheric compositions^{1,11,30}. For instance, in this geographical area, the isotope difference between Crozet¹⁵ and Marion-Prince Edward (S. R. Hart and A. J. Erlank, in preparation) could be interpreted either as resulting from completely different sources for these blobs or, conversely, as variable proportions of a common blob in the material erupted at the surface. More detailed studies of the Crozet swell are needed to elucidate this question.

Origin of the depleted end member. At the other extreme of the SWIR mixing trend, the depleted end member, considered as representative of the asthenosphere, is characterized by particularly nonradiogenic $^{206}\text{Pb}/^{204}\text{Pb}$ values, but higher $^{207}\text{Pb}/^{204}\text{Pb}$, $^{208}\text{Pb}/^{204}\text{Pb}$ and $^{87}\text{Sr}/^{86}\text{Sr}$ and lower $^{143}\text{Nd}/^{144}\text{Nd}$ ratios, compared with the lower end-member values of the MAR and EPR. It must be emphasized that, whereas several studies have already suggested some unexpected enrichment of Indian MORB^{11,15,31}, we present here the first evidence that this difference is indeed a regional characteristic of the most depleted end-member in this ocean.

The present results for the SWIR, the Dupré and Allègre¹⁵ data for the Carlsberg Ridge and our unpublished results on the East Indian Ridge now demonstrate fully that the whole Indian Ocean ridge system displays a characteristic isotope signature. Moreover, analogous Pb/Pb results have been found recently in the Xigaze ophiolites of Tibet³², which originated in the same geographical area 100-Myr ago. A geographical mapping of isotope compositions has already been discussed for oceanic island results^{15,33}. Our results described here demonstrate that similar regional variations also exist for the depleted upper-mantle value. Therefore, we conclude that the upper mantle itself must be divided in different provinces, which are probably related to different convective units. This raises the obvious question of the physical meaning of the different units and of the boundaries between them (for example, subduction planes, continents, transform faults).

Two issues are then to be discussed: first, to determine possible mechanisms for creating such an isotope composition, and second, to evaluate the consequences of such regional isotope units in asthenospheric reservoirs, all within the overall scheme of mantle convection.

Dupré and Allègre¹⁵ have argued that the specific isotope signature of Carlsberg MORB is a further demonstration of mixing between asthenosphere and blobs under ridges, and that the deviation of these samples from the main Atlantic-Pacific

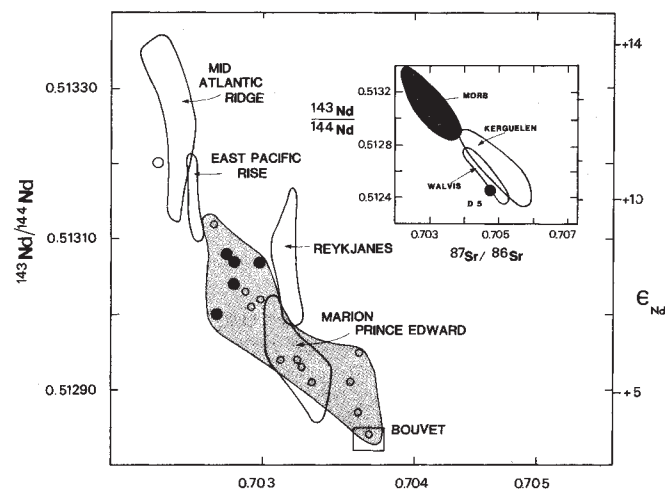


Fig. 4 Sr/Nd diagram. Open circles, data of Le Roex *et al.*¹⁷ for SWIR basalts in the region of Bouvet Island.

isotope trend results from the fact that the Indian Ocean islands have isotope characteristics removed from this trend. However, this model cannot explain the isotope composition of the depleted end-member itself, as there is no evidence from available data of any present-day mixing trend between the Atlantic-Pacific depleted end-member and an Indian Ocean island basalt contaminant. At the opposite extreme, the Indian depleted end-member seems to be fairly homogeneous. An additional mechanism, different from the present-day contamination of ridges by blobs discussed in the previous section, must then be proposed. This mechanism could involve a contamination of the whole upper mantle convection cell beneath the Indian Ocean by material with Kerguelen-type isotope values, if enough time has elapsed to allow the isotope homogenization of the contaminated reservoir.

For example, one could first advocate contamination of the upper mantle through reinjection of subducted oceanic lithosphere. The latter is first 'polluted' in accretion zones by blobs with Kerguelen-type signature, then later contaminates the whole upper mantle beneath the Indian Ocean, when it is mixed back after being subducted. This implies that the sub-Indian Ocean mantle has been isolated for several hundred Myr, and, moreover, that subduction-related material has been recycled continuously within the same convection cell. This model then seems unrealistic, especially taking into account the small number of subduction zones in the Indian Ocean. An alternative hypothesis could be that the upper mantle is contaminated by an ocean island basalt reservoir with Kerguelen signature through a 'diffusion-type' process; this contamination operates continuously and uniformly in solid state at depth in the mantle,

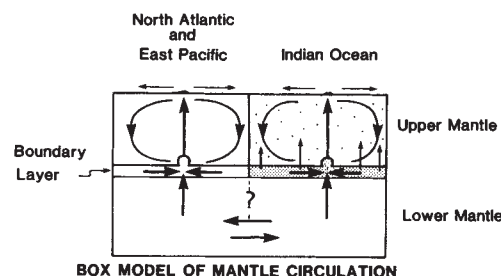


Fig. 5 Possible scheme of mantle dynamics. The upper mantle is divided in different discontinuous boxes, according to the SWIR MORB isotope results. The model where ocean island basalts originate in the boundary layer between upper and lower mantle³⁸ is illustrated as an example. Since the Indian Ocean island basalt source material (dotted area) is different from other parts of the world, the sub-Indian upper mantle is contaminated specifically by these Indian blobs, some of which reach the surface and others becoming progressively mixed within the upper mantle convection cell.

it allows isotopic homogenization within the upper mantle convection cell, but does not appear any more at the surface as ocean island volcanism, contrary to the present-day occurrence at ridges, for example, near Marion and Prince Edward islands. These different processes can operate simultaneously (Fig. 5); their relative importance depends on the respective scales and rates of mixing, reinjection and homogenization.

The final problem is the interpretation of the unusual values of the D5 dredge. At present, we can speculate using the duplicated analyses of the one sample recovered in that dredge; it will be of great interest to study the lateral extension of this anomaly. Similar results have not been found before on active ridges. On the other hand, the D5 composition is close to the lowest end-member of the Walvis Ridge, as discussed by Richardson *et al.*²², or to the nonradiogenic ilherzolite xenolith from Tanzania analysed by Cohen *et al.*³⁴. These results are slightly outside the oceanic mantle array³⁵ and are not easily interpretable as a mixing between blob-type material and typical depleted mantle values. Possible involvement in the genesis of these structures of material with continental isotope characteristics, through reinjection of sediments³⁶, continental lithosphere reassimilation^{34,37} or percolation of metasomatizing fluids²² should then be considered.

The MD34 cruise was organized with the efficient cooperation of the French Austral and Antarctic Territories. We thank the RV *Marion Dufresnes* crew for help during the mission. We also thank the scientific team and its chief scientist Ph. Patriat for their active collaboration in ridge exploration and sampling, B. Dupré for stimulating discussions, J. Patchett for comments and S. Richardson for help in preparation of the manuscript. This work was supported by the CNRS ATP 'Géologie-Géophysique des océans'. IGP contribution 795.

Received 19 October 1984; accepted 19 February 1985.

1. Tatsumoto, M. *Earth planet. Sci. Lett.* **38**, 63-87 (1978).
2. Allègre, C. J., Brévard, O., Dupré, B. & Minster, J. F. *Phil. Trans. R. Soc. A* **297**, 447-477 (1980).
3. Allègre, C. J., Hamelin, B. & Dupré, B. *Earth planet. Sci. Lett.* **71**, 71-84 (1984).
4. Hart, S. R., Schilling, J. G. & Powell, J. L. *Nature* **246**, 104-107 (1973).
5. Sun, S. S., Tatsumoto, M. & Schilling, J. G. *Science* **190**, 143-147 (1975).
6. White, W. M. & Schilling, J. G. *Geochim. cosmochim. Acta* **42**, 1501-1506 (1978).
7. Dupré, B. & Allègre, C. J. *Nature* **286**, 17-22 (1980).
8. Cohen, R. S., Evensen, N. M., Hamilton, P. J. & O'Nions, R. K. *Nature* **283**, 149-153 (1980).
9. Hamelin, B., Dupré, B. & Allègre, C. J. *Earth planet. Sci. Lett.* **67**, 340-350 (1984).
10. Church, S. E. & Tatsumoto, M. *Contr. Miner. Petrol.* **53**, 253-279 (1975).
11. Sun, S. S. *Phil. Trans. R. Soc. A* **297**, 409-445 (1980).
12. White, W. M. & Hofmann, A. W. *Yb. Carnegie Instn Wash.* **77**, 596-606 (1978).
13. Dupré, B. thesis, Univ. Paris VII (1983).
14. Dosso, L. *et al. Earth planet. Sci. Lett.* **43**, 46-60 (1979).
15. Dupré, B. & Allègre, C. J. *Nature* **303**, 142-146 (1983).
16. O'Nions, R. K., Hamilton, P. J. & Evensen, N. M. *Earth planet. Sci. Lett.* **34**, 13-22 (1977).
17. Le Roex, A. P. *et al. J. Petrology* **24**, 267-318 (1983).
18. Machado, N., Ludden, J. N., Brooks, C. J. & Thompson, G. *Nature* **295**, 226-228 (1982).
19. Manhès, G., Minster, J. F. & Allègre, C. J. *Earth planet. Sci. Lett.* **39**, 14-24 (1978).
20. Birk, J. L. & Allègre, C. J. *Earth planet. Sci. Lett.* **39**, 37-51 (1978).

21. Richard, P., Shimizu, N. & Allègre, C. J. *Earth planet. Sci. Lett.* **31**, 269-278 (1976).
22. Richardson, S. H., Erlank, A. J., Duncan, A. R. & Reid, D. L. *Earth planet. Sci. Lett.* **59**, 327-342 (1982).
23. Cohen, R. S. & O'Nions, R. K. *J. Petrology* **23**, 299-324 (1982).
24. Allègre, C. J., Montigny, R. & Bottinga, Y. *Bull. Soc. géol. Fr.* **7**, 461-477 (1973).
25. Jacobsen, S. B. & Wasserburg, G. J. *J. geophys. Res.* **84**, 7429-7445 (1979).
26. White, W. M. & Hofmann, A. W. *Nature* **296**, 821-825 (1982).
27. Zindler, A., Hart, S. R., Frey, F. A. & Jakobsson, S. P. *Earth planet. Sci. Lett.* **45**, 249-262 (1979).
28. Dosso, L. & Murthy, V. R. *Earth planet. Sci. Lett.* **48**, 268-276 (1980).
29. Verma, S. P. & Schilling, J. G. *J. geophys. Res.* **87**, 838-856 (1982).
30. Dupré, B., Lambret, B. & Allègre, C. J. *Nature* **299**, 620-622 (1982).
31. Frey, F. A., Dickey, J. S., Thompson, G., Bryan, W. B. & Davies, H. L. *Contr. Miner. Petrol.* **74**, 387-402 (1980).
32. Göpel, C., Allègre, C. J. & Rong-Hua Xu, *Earth planet. Sci. Lett.* **69**, 301-310 (1984).
33. Hart, S. R. *Nature* **309**, 753-756 (1984).
34. Cohen, R. S., O'Nions, R. K. & Dawson, J. B. *Earth planet. Sci. Lett.* **68**, 209-220 (1984).
35. Zindler, A., Jagoutz, E. & Goldstein, S. *Nature* **298**, 519-523 (1982).
36. Kurz, M., Jenkins, W. J. & Hart, S. R. *Nature* **297**, 43-47 (1982).
37. McKenzie, D. & O'Nions, R. K. *Nature* **301**, 229-231 (1983).
38. Allègre, C. J. & Turcotte, D. L. *EOS* **65**, 296 (1984).

A short conserved sequence is involved in the light-inducibility of a gene encoding ribulose 1,5-bisphosphate carboxylase small subunit of pea

Giorgio Morelli*, Ferenc Nagy*, Robert T. Fraley†, Stephen G. Rogers† & Nam-Hai Chua*

* Laboratory of Plant Molecular Biology, Rockefeller University, 1230 York Avenue, New York, New York 10021-6399, USA

† Monsanto Company, 800 N. Lindbergh Boulevard, St Louis, Missouri 63167, USA

Light induction of chloroplast maturation in plant seedlings ('greening') is associated with the induction in expression of the enzyme ribulose 1,5-bisphosphate carboxylase. To define the regions of a gene encoding the small subunit of ribulose 1,5-bisphosphate carboxylase involved in light inducibility of pea plants, we have introduced derivatives of the gene into petunia cells using a tumour-inducing plasmid vector. A conserved 33-base pair sequence close to the 'TATA' box of the gene is sufficient to confer light inducibility of the small subunit gene.

LIGHT has profound morphogenetic effects on chloroplast development. Seedlings germinated in the dark are arrested in plastid development at the etioplast stage that can then be converted to chloroplasts on illumination. During etioplast-chloroplast transformation there is a rapid synthesis of chlorophylls and a dramatic increase in thylakoid membrane polypeptides and stromal proteins¹. Although the molecular mechanisms by which these biosynthetic events are turned on by light are still unknown, an important regulatory stage is probably the transcription control of genes encoding chloroplast proteins.

The most abundant protein that increases in concentration during greening is the stromal enzyme ribulose 1,5-bisphosphate carboxylase/oxygenase. This enzyme complex, which accounts for $\leq 50\%$ of soluble protein in plants, is composed of eight copies each of two non-identical subunits². The large subunit (rbcL) is encoded in the chloroplast³, whereas the small subunit (rbcS) is encoded in the nucleus by a small multigene family⁴⁻⁸. In peas, the amount of rbcS polypeptide in green leaves is 100 times that in etiolated leaves. The increase in rbcS polypeptide during greening is followed by a concomitant increase in the steady-state messenger RNA level⁹⁻¹¹ so that in fully greened leaves, the *rbcS* transcript becomes the most abundant mRNA in mesophyll cells¹². The light-stimulated increase in *rbcS* mRNA level in peas, as well as other higher plants, has been shown to be mediated by phytochrome¹³⁻¹⁵. There is evidence that the control of *rbcS* gene expression by light¹⁶ or phytochrome¹⁷ is at the transcriptional level.

We are interested in defining the molecular events surrounding light-regulated expression of plant nuclear genes. For our investigations we have selected one member (*rbcS-E9*) of the pea *rbcS* gene family as a model system, because the nucleotide sequence of this gene is known and its expression in different tissues of green and etiolated pea plants has been well characterized¹¹. Our first objective is to define DNA sequence elements needed for its light/dark regulation and efficient transcription. Previously, we transferred the pea *rbcS-E9* gene into petunia via a tumour-inducing (Ti)-mediated gene transfer vector¹⁸ and demonstrated that this gene is transcribed accurately using its own promoter¹⁹. Moreover, the *rbcS-E9* mRNA level is regulated by light in petunia calli as it is in pea leaves. These results provide evidence that the petunia expression system can be used to characterize the control sequences of the *rbcS-E9* gene. Our confidence in the fidelity of heterologous expression systems is reinforced by the finding that the 5' flanking region of another pea *rbcS* gene (*ss3.6*) also shows light-regulated expression after transfer into green tobacco calli²⁰.

Here we have examined the expression of 5' deletion mutants of *rbcS-E9* and chimaeric genes in petunia calli. We have iden-

tified an upstream region needed for maximal expression and delimited a light-responsive sequence to a 33-base pair (bp) region around the TATA box.

Internal deletion and 5' mutants

With a few exceptions^{21,22}, most of the animal genes transcribed by RNA polymerase II contain regulatory elements upstream of their transcription start sites²³. Therefore, we investigated first the role of 5' upstream sequences in the regulation of *rbcS-E9* gene expression. Previously¹⁹, we demonstrated that a *rbcS-E9* gene (*rbcS-E9 5' Δ-1052*) containing 1,052 bp of 5' upstream sequence was transcribed accurately in petunia calli in a light-regulated manner. This gene, therefore, was chosen as the wild type and from this a series of 5' deletion mutants were constructed by *Bal31* nuclease digestion (Fig. 1). The deletion mutants were first screened by gel electrophoresis and those in the desired size range were characterized further by sequence analysis to determine their end points. A total of seven 5' deletion mutants with end points at strategic locations in the 5' region were chosen for the present study. The first mutant (*rbcS-E9 5' Δ-437*) contains a deletion of ~600 bp; the next three (*rbcS-E9 5' Δ-352*; *rbcS-E9 5' Δ-255*; *rbcS-E9 5' Δ-96*) have end points spaced ~100 bp apart; the next (*rbcS-E9 5' Δ-87*) has its end point just 5' of the CAAT box; one (*rbcS-E9 Δ-35*) has its end point between the CAAT box and the TATA box, and the remaining mutant (*rbcS-E9 5' Δ-13*) contains neither the CAAT box nor the TATA box and retains only 13 bp upstream of the transcription start site. In addition, we have exploited two convenient *Bst*XI sites flanking the CAAT box to construct a mutant with an internal deletion of 51 bp from -107 to -56.

The internal deletion mutant (*rbcS-E9 5' Δ-107/-56*) and the 5' deletion mutants were transferred into petunia cells via the Ti-plasmid of *Agrobacterium tumefaciens*, using the pMON145 vector for plant gene transfer. This vector contains the nopaline synthase (*nos*)-neomycin phosphotransferase (*npt*)-II chimaeric gene that confers resistance to kanamycin¹⁸. Transformed calli were selected and maintained on hormone-free medium containing kanamycin (100 $\mu\text{g ml}^{-1}$) in continuous light or kept in complete darkness for 5 days before RNA extraction and analysis. To eliminate any possible variations in the physiological state of the calli or in the purity of poly(A)⁺ RNA among samples, we have used the level of the *npt*-II mRNA transcribed from the *nos* promoter as an internal standard. The use of an internal control also enabled us to reduce the variation resulting from different copy numbers of integrated tumour(T)-DNA, which contained the two genes (*rbcS-E9* and *nos-npt*-II) linked together. Furthermore, calli from different transformation events were pooled to minimize any possible position effects on expression. Transcriptional activities of the mutants were

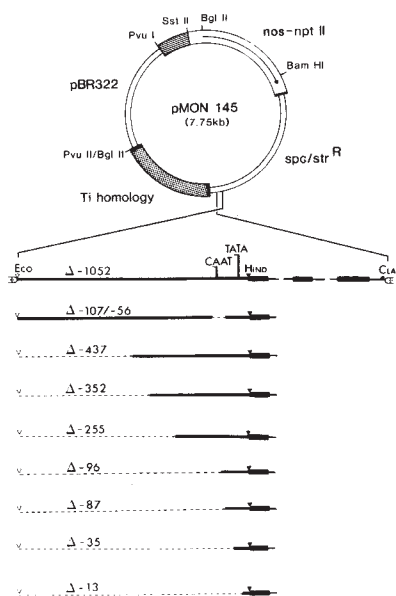


Fig. 1 Construction of *rbcS-E9* deletion mutants. The pea *rbcS-E9* gene is encoded by a 2.4-kb *EcoRI*/*ClaI* fragment that has 1,052 bp of DNA sequence upstream from the transcriptional start site¹¹. This fragment was cloned between the *EcoRI* and *ClaI* site of pBR325 to give the parental plasmid, pE9. (A restriction map of the 2.4-kb *EcoRI*/*ClaI* fragment is shown in Fig. 3.) To generate 5' deletion mutants, pE9 was linearized at the unique *EcoRI* site and digested with *Bal31* (ref. 36); the digested DNA molecules were ligated to *EcoRI* linkers (CGGAATTCCG; New England Biolabs), treated with *EcoRI* and then recircularized by *T₄* ligase. The resulting series of 5' deletion mutants was analysed by agarose gel electrophoresis to determine the extent of deletion and selected mutants were further characterized by sequence analysis according to Maxam and Gilbert³⁷. Mutants with end points at -437, -352, -255, -96, -87, -35 and -13 were selected for subsequent experiments. To generate the internal deletion mutants (*rbcS* 5' Δ -107/-56), pE9 was digested with *BstXI* (CAANNNNNTTGG), which cuts the promoter region of the *rbcS-E9* gene at -56 and -107 (ref. 11). The staggered ends of the deleted plasmids were repaired with *T₄* DNA polymerase and the DNA was then recircularized by *T₄* DNA ligase. The promoter fragment (*EcoRI*/*HindIII*) of the internal deletion mutant (*rbcS* 5' Δ -107/-56) was subcloned with the *EcoRI* and *HindIII* sites of M13 mp8 and the break points determined by dideoxy sequence analysis³⁸. The parental gene pE9(5' Δ -1052), the 5' deleted mutants (5' Δ -437, and so on) and the internal deletion mutants (5' Δ -107/-56) were subcloned into the unique *EcoRI* and *ClaI* restriction sites of the intermediate vector, pMON145 (ref. 19). The intermediate vector and its derivatives containing the different *rbcS-E9* deletion mutants in *Escherichia coli* (LE392) were mobilized into an octopine strain of *A. tumefaciens* (GV3111) by triparental mating and the co-integrates selected according to Fraley *et al.*¹⁸.

assessed by their relative transcript level, which is the ratio of *rbcS-E9* mRNA to the *nos-npt-II* mRNA. Poly(A)⁺ RNAs from pea leaves and from pMON145 calli were analysed along with the mutant samples to localize the positions of the *nos-npt-II* and pea *rbcS-E9* transcripts.

Figure 2 shows that in the hybridization and washing conditions used, poly(A)⁺ RNA from the gene transfer vector, pMON145, gives only one radioactive band on Northern blots, corresponding in size to the *nos-npt-II* transcript. No cross-hybridization of the pea *rbcS-E9* probe to the petunia *rbcS* mRNA, which is identical in size to the *rbcS-E9* mRNA, was detected. In agreement with previous results¹⁹, we found that the wild-type construct (*rbcS-E9* 5' Δ -1052) produces about five times as much *rbcS-E9* mRNA compared with *nos-npt-II* mRNA (Fig. 2). Deletion of 615 bp from the 5' upstream region of *rbcS-E9* 5' Δ -1052 decreases the relative transcript level by about three times (mutant *rbcS-E9* 5' Δ -437) and an additional

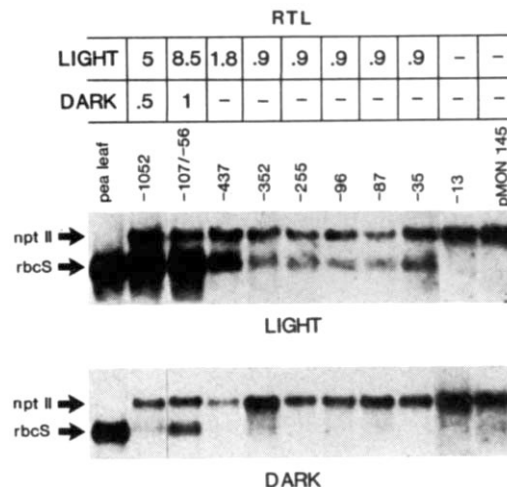


Fig. 2 Northern blot analysis of deletion mutants. The extreme right lane contains 1 μ g poly(A)⁺ RNA from pMON145 calli, whereas the extreme left lane contains 60 ng poly(A)⁺ RNA from pea leaves. The remaining lanes, indicated by numbers, contain 1 μ g poly(A)⁺ RNA from each of the deletion mutants. The relative transcript level (RTL) refers to the ratio of the *rbcS-E9* transcript to the *nos-npt-II* transcript (upper panel).

Methods. Petunia cells were transformed with *A. tumefaciens* by co-cultivation¹⁸. Calli obtained from transformation with the pMON145 vector alone or the vector containing the various deletion mutants were propagated on 1% agar plates containing Murashige and Skoog medium and 100 μ g ml⁻¹ kanamycin¹⁹. Tissues were maintained in continuous light or transferred to total darkness for 5 days before collecting. Total RNA was isolated with guanidinium thiocyanate as a protein denaturant³⁹ and poly(A)⁺ RNA was purified by chromatography on poly(U)-Sepharex⁴⁰. Aliquots of RNA were denatured in glyoxal at 50 °C⁴¹, electrophoresed on 1% agarose gels and transferred onto nitrocellulose filters⁴². Filters were hybridized to ³²P-labelled probes containing the coding sequence of the *nos-npt-II* chimaeric gene and of the *rbcS-E9* gene using the hybridization and washing conditions described previously^{11,19}. Radioactive bands on Northern blots were visualized by autoradiography and quantified according to Suissa⁴³.

deletion of 85 bp reduces the relative transcript level further by about twofold (mutant *rbcS-E9* 5' Δ -352). Sequences between -352 and -35, which include the CCAAT box, have little effect on the relative transcript level, whereas no *rbcS-E9* mRNA was detected in the mutant *rbcS-E9* 5' Δ -13 that has the entire 5' upstream region, including the TATA box, deleted. From these results, three conclusions can be drawn: (1) a 700-bp sequence between -1,052 and -352 of the *rbcS-E9* upstream region modulates the relative transcript level about six times; (2) The CAAT box centred around -85 does not change the level; and (3) The TATA box is needed for transcription of the *rbcS-E9* gene.

To collect further evidence on the function of the CAAT box, we made use of two convenient *BstXI* sites to generate a mutant (*rbcS-E9* 5' Δ -107/-56) with an internal deletion from -107 to -56. The deleted segment contains the CAAT box with ~50 bp of surrounding sequences. Figure 2 (upper panel) shows that the mutant *rbcS-E9* 5' Δ -107/-56 is expressed in light-grown calli with a relative transcript level of about twice that of the wild type. This result provides unequivocal evidence that the CAAT box is not required for the expression of the *rbcS-E9* gene in petunia calli.

In an attempt to localize DNA sequences required for light-regulated expression of the *rbcS-E9* gene, transformed petunia calli carrying the different deletion mutants were transferred to the dark for 5 days before their RNAs were extracted for Northern blot analysis. The wild-type construct produces a reduced level of *rbcS-E9* mRNA in the dark compared with the light (Fig. 2, lower panel), confirming previous observations¹⁹. We

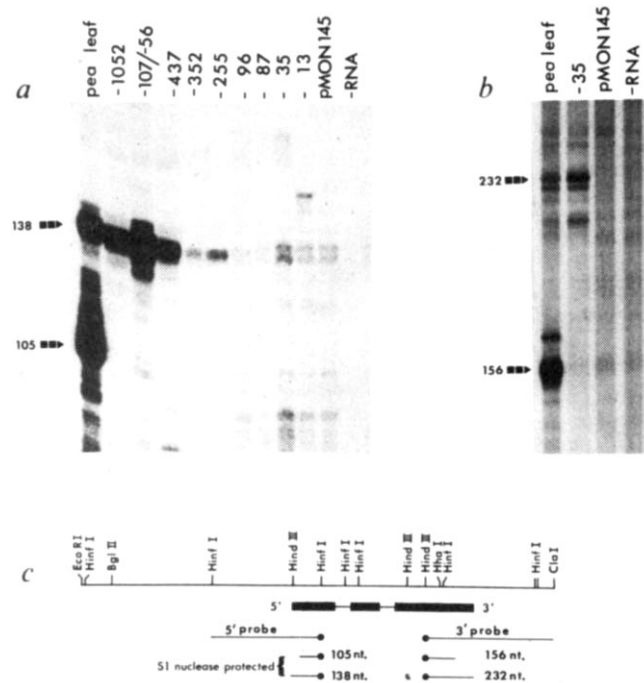


Fig. 3 S_1 nuclease protection assays of pea *rbcS* transcripts from the deletion mutants. Poly(A)⁺ RNAs from both pea leaf and the deletion mutants were probed with the 541-nucleotide *HinfI*/*HinfI* fragment (5' probe; c, left) or with the 690-nucleotide *HindIII*/*Clal* fragment (3' probe; c, right). **a**, Pea-leaf poly(A)⁺ RNA, 60 ng; poly(A)⁺ RNA from *rbcS-E9 5' Δ-1052*, 40 ng; poly(A)⁺ RNA from the other deletion mutants, 1 μg. Arrow 138 indicates the end and the length of the 5' protected fragment. **b**, Pea-leaf poly(A)⁺ RNA, 60 ng; *rbcS-E9 5' Δ-35* poly(A)⁺ RNA, 10 μg; pMON145 poly(A)⁺ RNA, 10 μg. Arrow 232 indicates the end and the length of the 3' protected fragment. Pea-leaf RNAs give two additional S_1 signals at the 5' (**a**, arrow 105) and 3' (**b**, arrow 156) ends. These result from limited hybridization of other pea *rbcS* transcripts to the probe^{11,19}.

Methods. S_1 nuclease protection assays were performed according to Berk and Sharp⁴⁴ as modified by Weaver and Weissman⁴⁵. The 5' probe is the 541-nucleotide *HinfI*/*HinfI* fragment, whereas the 3' probe is the 690-nucleotide *HindIII*/*Clal* fragment (lower panel). The ³²P-DNA fragment labelled at either their 5' or 3' ends were denatured and the dissociated strands separated by polyacrylamide gel electrophoresis. The isolated strands were hybridized with poly(A)⁺ mRNA in 10 μl of a solution containing 50% formamide, 0.4 NaCl, 2 mM EDTA and 20 mM PIPES (pH 6.8) for 12 h at 42 °C. After hybridization, the reaction mixture was diluted to 300 μl with a solution containing 0.3 M NaCl, 30 mM NaOAc (pH 4.6), 1 mM ZnSO₄, 20 μg ml⁻¹ denatured salmon sperm DNA, 300 U ml⁻¹ S_1 nuclease and incubated at 37 °C for 45 min. DNA fragments protected from S_1 nuclease digestion were sized on 6% sequencing gels and visualized by autoradiography.

estimated that the relative transcript level of the dark sample is ~0.5, giving a light-induction factor of ~10 for the *rbcS-E9* mRNA. None of the 5' deletion mutants (*rbcS-E9 5' Δ-437*, *Δ-352*, *Δ-255*, *Δ-96*, *Δ-87* and *Δ-35*) active in the light produce detectable amounts of *rbcS-E9* mRNA in the dark. The extremely low level of *rbcS-E9* mRNA in the dark has prevented the estimation of the light-induction factor for the deletion mutants. Nevertheless, it is clear from our results that the mutant, which contains only 35 bp of sequences 5' to the transcription start site, is still capable of light-regulated expression.

The internal deletion mutant (*rbcS-E9 5' Δ-107/-56*) produces a higher level of *rbcS-E9* mRNA in the dark compared with the wild type (Fig. 1, lower panel), with a dark relative transcript level ~1. Because this mutant is also more active in the light, its light-induction factor is about the same as that of wild type. The light-regulated expression of this mutant provides direct

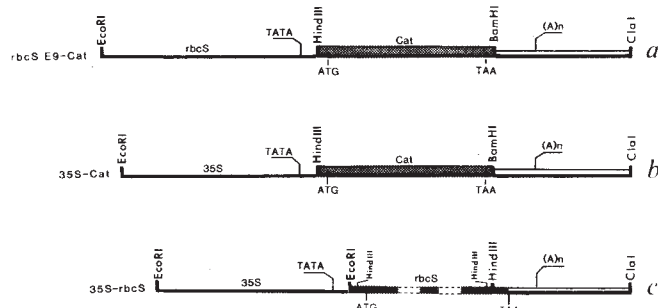


Fig. 4 Construction of chimaeric genes. **a**, *rbcS-E9-cat*. The *HindIII*/*Sau3A* fragment coding for CAT in pSV2-cat(24) was subcloned into the *HindIII*/*Bam*HI sites of pUC12 to give pUC-cat. The 699-bp *HindIII*/*HindIII* fragment containing the 3' end of *rbcS-E9* (ref. 11) was then subcloned into the *Bam*HI site of pUC-cat giving pUC-cat 3' *rbcS*. The *rbcS-E9* gene was subcloned in between the *Eco*RI and *Clal* site of a *HindIII* minus a derivative of pMON145 (*rbcS-E9 5' Δ-1052*). The *rbcS-E9* coding sequence from *HindIII* (-2) to *Clal* (+1,349) was replaced by the 1,549-bp *HindIII*/*Clal* fragment from pUC-cat 3' *rbcS*. The resulting chimaeric genes were designated *rbcS-E9-cat*. The 1,052-bp *Eco*RI/*HindIII* fragment of *rbcS-E9-cat*, containing the *rbcS-E9 5' flanking* sequence, was replaced by a 950-bp *Eco*RI/*HindIII* fragment containing the 35S promoter of CaMV²⁵. **c**, ³⁵S-promoter-*rbcS*. The 950-bp *Eco*RI/*HindIII* containing the 35S promoter was cloned into the unique *Eco*RI site of *rbcS-E9 5' Δ-13*, using *Eco*RI linkers. The thick line between *HindIII* and *Bam*HI indicates the *cat* coding sequence (**a**, **b**). The white line between *Bam*HI and *Clal* (**a**, **b**) or *HindIII*/*Clal* (**c**) indicates the 3' end of *rbcS-E9*. The marked line between *Eco*RI and *HindIII* indicates the *rbcS* coding sequence.

evidence that the 5' sequence region from -107 to -56, including the CAAT box, is not required for light responsiveness of the *rbcS-E9* gene.

5' and 3' S_1 analyses

We have reported previously that a *rbcS-E9* gene containing 1,052 bp of 5' upstream sequence is transcribed with the correct start site in petunia calli¹⁹. To examine the effects of upstream sequences on transcriptional initiation, we subjected poly(A)⁺ RNAs from the different deletion mutants to 5' S_1 nuclease protection assays. Figure 3a shows that pea leaf poly(A)⁺ RNA gives two S_1 -protected fragments, using a probe that covers the 5' end of *rbcS-E9*. The longer fragment is produced by hybridization of the *rbcS-E9* probe with its cognate mRNA, whereas the shorter fragment is the result of hybridization by other pea *rbcS* transcripts that show sequence homology to the probe up to the first AUG¹¹. Consistent with this interpretation, RNA from petunia calli transformed with *rbcS-E9 5' Δ-1052* gives only the longer fragment, indicating that the correct transcription start site is used. Deletion of the 5' upstream sequence from -1,052 to -255 does not change the S_1 start site. We were unable to obtain reproducible S_1 signals for the remaining 5' deletion mutants because of the low amount of *rbcS-E9* mRNA, but an internal deletion between -107 to -56 also has no effect. Taken together, these results demonstrate that the sequence elements surrounding the CAAT box as well as DNA sequences upstream of -255 do not affect the accuracy of transcriptional initiation.

The polyadenylation site of the pea *rbcS-E9* gene has been mapped previously to 150 bp downstream from the termination codon¹¹. To see whether the same site on the *rbcS-E9* gene of pea is also recognized in petunia calli, poly(A)⁺ RNAs from *rbcS-E9 5' Δ-35* were subjected to S_1 analysis using a probe that covers the 3' region of *rbcS-E9*. Figure 3 shows that identical polyadenylation sites are used in petunia and pea, indicating conservation of this processing signal in both plant species.

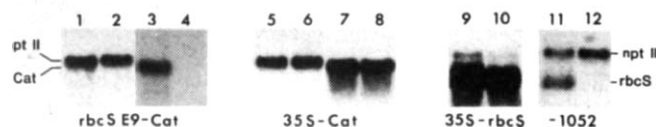


Fig. 5 Northern blot analysis of chimaeric genes. All lanes contain 1 µg poly(A)⁺ RNA from: *rbcS-E9-cat* light-grown (lanes 1, 3) and dark-grown calli (lanes 2, 4); from 35S promoter-*cat* light-grown (lanes 5, 7) and dark-grown calli (lanes 6, 8); from 35S promoter-*rbcS* light-grown (lane 9) and dark-grown calli (lane 10); from *rbcS-E9* 5' Δ-1052 light-grown (lane 11) and dark-grown calli (lane 12). The filters were hybridized to the following ³²P-nick translated probe: *npt-II* coding sequence (lanes 1, 2, 5, 6); *cat* coding sequence (lanes 3, 4, 7, 8); *npt-II* coding sequence plus 3' end of *rbcS-E9* (lanes 9-12). (Conditions for transformation and growth of the transformed calli and isolation and analysis of the RNAs are described in Fig. 2.)

Expression of chimaeric genes

Our results with the 5' deletion mutants have allowed us to place the 5' boundary of the light-responsive sequence of *rbcS-E9* at -35. To determine the 3' limit of this sequence, we used a convenient *Hind*III site near the S₁ start site of *rbcS-E9*. An *Eco*RI/*Hind*III fragment containing the 5' region (from -1,052 to -2) of *rbcS-E9* was fused to the coding sequence of bacterial chloramphenicol acetyltransferase (*cat*)²⁴ followed by the 3' polyadenylation sequence of *rbcS-E9* (Fig. 4). We found that this chimaeric gene is expressed in petunia calli; moreover, its activity is regulated by light (Fig. 5, lanes 3, 4). Control experiments showed that the *nos-npt-II* mRNA is expressed equally in the light and dark (Fig. 5, lanes 1, 2). The light/dark regulation of the *cat* mRNA is abolished when the *rbcS-E9* promoter fragment is replaced with a 35S promoter fragment (from -941 to +9) of cauliflower mosaic virus (CaMV) (Fig. 5, lanes 7, 8), which is known to be expressed constitutively²⁵. Because the second chimaeric gene contains the 3' polyadenylation sequence of *rbcS-E9* (Fig. 4), these results indicate that the sequence, by itself, does not have a direct role in light response. In addition, the results also establish that the 5' upstream sequence of *rbcS-E9*, from -1,052 to -2, is sufficient to confer light-regulated transcription, thus placing the 3' boundary of the light-responsive sequence at position -2. Our results with the *rbcS-E9* promoter fragment are in agreement with the previous report of Herrera-Estrella *et al.*²⁰ who used a similar 5' flanking fragment of another pea *rbcS* gene (*ss3.6*).

In the experiments described above, the *npt-II* and the *cat* mRNA could not be resolved on the same Northern blot because of their size similarity. Therefore, the two transcripts were probed separately. The *npt-II* hybridization signal was obtained after 24 h exposure of the X-ray film (Fig. 5, lanes 1, 2) whereas that for the *cat* mRNA required an exposure time of 7 days (Fig. 5, lanes 3, 4). These results, compared with those in Fig. 2, strongly suggest that the *cat* mRNA level is much lower than that for the *rbcS-E9* mRNA when driven by the same promoter of *rbcS-E9*. The poor expression of the *rbcS-E9* 5' flanking sequence when joined to a heterologous coding sequence suggests a role for the *rbcS-E9* intragenic region in transcription.

Although we have shown that the *rbcS-E9* 5' flanking sequence (-1,052 to -2) of pea is able to drive the light-regulated transcription of a test gene, it is possible that differential stability of the *rbcS-E9* mRNA may also contribute to the observed differences in *rbcS-E9* mRNA accumulation in the light and dark. To examine this possibility we used the CaMV 35S promoter fragment²⁴ to drive the expression of the *rbcS-E9* coding sequence. Figure 5 (lanes 9, 10) shows that the CaMV 35S promoter-*rbcS* hybrid gene is expressed equally in the light and dark, as is the case with *nos-npt-II*, which has been shown previously to be expressed constitutively²⁵. Control experiments confirm the expected difference in the *rbcS-E9* mRNA levels between light and dark when the coding sequence is driven by

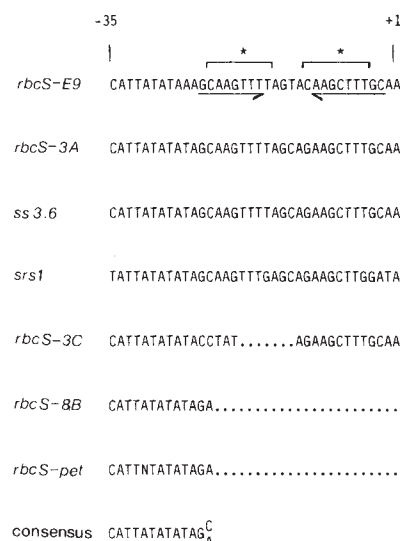


Fig. 6 Comparison of sequences between the TATA box and the transcriptional start site of several *rbcS* genes. Dicotyledonous gene sequences were obtained from the following authors: *Pisum sativum rbcS-E9*¹¹; *P. sativum rbcS-3A* (P. Moses, personal communication); *P. sativum ss3.6*⁴⁶; *Glycine max srs1 max*⁴; *P. sativum rbcS-3C* (P. Moses, personal communication); *Nicotiana plumbaginifolia rbcS-8B* (C. Poulsen, personal communication); *Petunia hybrida rbcS-pet* (Monsanto Company, personal communication). Parentheses, direct repeats found in *rbcS-E9*; asterisks, mismatch in the repeat; arrows underline the imperfect palindromic sequence. Sequence downstream of the *rbcS-3C* TATA box are aligned with those of *rbcS-E9* to maximize homology. Similar sequences are not shown for *rbcS-8B* and *rbcS-pet* because of the lack of obvious homology. A consensus sequence derived from the TATA box for the various *rbcS* genes is given.

its cognate promoter (Fig. 5, lanes 11, 12). These results provide strong evidence that light has no effect on the *rbcS-E9* transcript processing or mRNA stability. The light-regulated expression of the *rbcS-E9* gene and its mutant derivatives in pea, therefore, mainly reflect control at the transcriptional level.

The experiments described above provide information on the relative strength of the CaMV 35S promoter and the *rbcS-E9* promoter. In these experiments, the Northern blots were hybridized with a probe containing a 1.2-kilobase (kb) coding sequence of *npt-II* joined to a 0.69-kb 3' sequence from the *Hind*III (+660) to the *Cla*I (+1,350) of the *rbcS-E9* gene¹¹. The *rbcS-E9* 3' fragment contains only 232 bases in the transcribed region that could hybridize to the *rbcS-E9* mRNA. In these conditions, the *nos-npt-II* and *rbcS-E9* mRNA show the same hybridization signal (Fig. 5, lane 11). However, the hybridization intensity of the *rbcS-E9* transcript produced by the CaMV 35S promoter relative to that of *npt-II* produced by the *nos* promoter is ~10-15 times higher (Fig. 5, lane 9). These results indicate that the CaMV 35S promoter is ~10-15 times stronger than the *rbcS-E9* promoter in petunia calli.

Discussion

Here we describe the first functional analysis of the control sequences of a major regulated plant gene, the *rbcS-E9* gene of pea. In pea leaves¹¹ and in transformed petunia calli¹⁹ the accumulation of the *rbcS-E9* mRNA is increased by light. Gallagher and Ellis¹⁶ have shown that an effect of light is to stimulate the rate of transcription initiation of the pea *rbcS* genes. However, the extent of light-stimulated transcription obtained by these authors¹⁶ is twofold less than the *rbcS* mRNA ratio between green and etiolated pea leaves^{9,11}. To determine whether light also exerts a post-transcriptional effect on *rbcS* gene

expression, we placed the *rbcS-E9* coding sequence under the control of the 35S promoter of CaMV²⁵ that is insensitive to light (F.N., unpublished results). The level of the *rbcS-E9* transcript produced by the 35S promoter is the same either in the light or the dark. These results clearly exclude any pronounced light effect on post-transcriptional processing of the *rbcS-E9* transcript or its stability. In addition, they provide evidence that light-induced accumulation of the *rbcS-E9* mRNA in petunia calli reflects largely transcriptional control of its cognate promoter by light.

To map the *rbcS-E9* gene control sequences, the activities of several 5' deletion mutants and chimaeric gene constructs were examined. Our analyses reveal that the *rbcS-E9* 5' flanking sequence can be divided roughly into three regulatory regions. An upstream region of ~700 bp (–1,052 to –352) is needed for maximal transcription but not light-dark regulation. Mutants lacking this region show 15–20% of the wild-type activity but still retain light responsiveness. Whether this region alone may also provide a light-regulated function when joined to a heterologous promoter is being tested.

The sequence element, bordered by positions –107 to –56 and including the CAAT box at position –85, is required neither for accurate transcription nor for light induction. In fact, this element seems to have a negative regulatory function, because in its absence transcription is increased by approximately two times with no significant effect on the light-induction factor. This finding is in contrast to the results obtained with the nopaline synthase promoter²⁶ and the CaMV 35S promoter²⁵, both of which require sequences in and around the CAAT box for efficient transcription. On the other hand, there are examples of animal genes that either do not have recognizable CAAT sequence^{27–30} or still retain full transcriptional activities after deletion of the CAAT sequence^{31,32}. We note that in the internal deletion mutant 5' Δ -107/-56, a new sequence, AATCCATT, is reconstituted at ~position 60 on re-ligation of the *Bst*XI-cut sites. Although unlikely, we cannot exclude the possibility that the function of the CAAT box at position –85 may be supplanted by this new sequence at position –60 in the deletion mutant.

We have identified a 33-bp sequence fragment surrounding the TATA box involved in light control of *rbcS-E9* gene transcription based on analyses of the *rbcS-E9-cat* chimaeric gene and the 5' Δ -35 mutant, both of which show light-induced tran-

scription. The chimaeric gene contains the *rbcS-E9* 5' region from –1,052 to –2. On the other hand, results from the 5' Δ -35 mutant clearly demonstrate that sequences upstream of –35 are not needed for light-induced transcription. Taken together, we conclude that the sequence from –35 to –2, which includes the TATA box, contains the necessary information for responsiveness to light.

Comparison of the 10-bp sequence (–35 to –24) containing the *rbcS-E9* TATA box with similar regions of all the *rbcS* genes of dicotyledonous plants sequenced so far reveals a striking degree of homology (Fig. 6). Point mutations or deletions in the TATA box are known to reduce the efficiency and accuracy of transcription but have no effect on gene regulation^{31,33}. In heat-shock³⁴, metallothionein³⁰ and His³⁵ genes, sequence elements that confer regulated transcription are found as short direct or inverted repeats 5' upstream of the TATA box. Examination of the *rbcS-E9* light-responsive sequence reveals an 8-bp direct repeat (from –23 to –16 and from –12 to –5) with only one mismatch (at positions –19 and –8) located just downstream of the TATA box. In addition, this region also contains an imperfect palindrome (Fig. 6). Although the functional significance of these structural features remains to be elucidated, we note that the sequence between the *rbcS-E9* TATA box and its mRNA cap site is conserved in two other pea *rbcS* genes (*rbcS-3A* and *ss3.6*) and in a soybean *rbcS* gene (*srs1*). A fourth pea *rbcS* gene, *rbcS-3C*, contains only the second repeat sequence of *rbcS-E9* (Table 1). The CaMV 35S promoter-*rbcS* chimaeric gene contains one (from –13 to –2) of the repeat sequences; nevertheless, it is not light-regulated (Fig. 5), suggesting that one of the repeat sequences by itself is not sufficient to confer light responsiveness. 3' deletion and site-specific mutagenesis in this region will allow us to investigate the relative importance of the TATA box and the direct repeats, and to define more precisely the critical sequences for light-controlled transcription.

We thank Cynthia L. Fink, Mei-Yin Hsu, Jeanne G. Niedermeyer, Karen Thurman, Sara Trillo Adams and Sara Wagner for technical assistance and Wendy Roine for assembling this manuscript. G.M. is a Fellow of the Instituto Pasteur-Fondazione Cenci Bolognietti (University of Rome) and F.N. is on leave from the Biological Research Center, Hungarian Academy of Science, Szeged, Hungary. Work done at the Rockefeller University was supported by a grant from Monsanto.

Received 24 January; accepted 19 March 1985.

- Virgin, H. I. & Egneus, H. *Encyclopedia of Plant Physiology* **16A**, 289–311 (1983).
- Ellis, R. J. *Trends biochem. Sci.* **11**, 241–244 (1979).
- Coen, D. M., Bedbrook, J. R., Bogorad, L. & Rich, A. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5487–5491 (1977).
- Berry-Lowe, S. L., McKnight, T. D., Shah, D. M. & Meagher, R. B. *J. molec. appl. Genet.* **1**, 483–498 (1982).
- Coruzzi, G., Broglie, R., Cashmore, A. R. & Chua, N.-H. *J. biol. Chem.* **258**, 2399–2402 (1983).
- Broglie, R., Coruzzi, G., Lampka, G., Keith, B. & Chua, N.-H. *Biotechnology* **1**, 55–61 (1983).
- Dunsmuir, P., Smith, S. & Bedbrook, J. *Nucleic Acids Res.* **11**, 4177–4873 (1983).
- Wimpee, C. F., Stiekema, W. J. & Tobin, E. M. in *Plant Molecular Biology* (ed. Goldberg, R. B.) 391–401 (Liss, New York, 1983).
- Smith, S. M. & Ellis, R. J. *J. molec. appl. Genet.* **1**, 127–137 (1981).
- Jenkins, G. I., Hartley, M. R. & Bennett, J. *Phil. Trans. R. Soc. B303*, 419–431 (1983).
- Coruzzi, G., Broglie, R., Edwards, C. & Chua, N.-H. *EMBO J.* **3**, 1671–1679 (1984).
- Broglie, R., Bellemare, G., Bartlett, S. G., Chua, N.-H. & Cashmore, A. R. *Proc. natn. Acad. Sci. U.S.A.* **78**, 7304–7308 (1981).
- Tobin, E. M. *Pl. molec. Biol.* **1**, 35–51 (1981).
- Sasaki, Y., Sakihama, T., Kamikubo, T. & Shinozaki, K. *Eur. J. Biochem.* **133**, 617–620 (1983).
- Thompson, W. F., Everett, M., Polans, N. O., Jorgensen, R. A. & Palmer, J. D. *Planta* **158**, 487–500 (1983).
- Gallagher, T. F. & Ellis, R. J. *EMBO J.* **1**, 1493–1498 (1982).
- Silverthorne, J. & Tobin, E. M. *Proc. natn. Acad. Sci. U.S.A.* **81**, 1112–1116 (1984).
- Fraleigh, R. T. *et al. Proc. natn. Acad. Sci. U.S.A.* **80**, 4803–4807 (1983).
- Broglie, R. *et al. Science* **224**, 838–843 (1984).
- Herrera-Estrella, L. *et al. Nature* **310**, 115–120 (1984).
- Banerji, J., Olson, L. & Schaffner, W. *Cell* **33**, 729–740 (1983).
- Charnay, P. *et al. Cell* **38**, 251–263 (1984).

- Breathnach, R. & Chambon, P. *Rev. Biochem.* **50**, 349–384 (1981).
- Gorman, C. M., Moffat, L. F. & Howard, B. H. *Molec. cell. Biol.* **2**, 1044–1051 (1982).
- Odell, J. T., Nagy, F. & Chua, N.-H. *Nature* **313**, 810–812 (1985).
- Shaw, C. H., Carter, G. H., Watson, M. D. & Shaw, C. H. *Nucleic Acids Res.* **12**, 7831–7846 (1984).
- Holmgren, R., Corces, V., Morimoto, R., Blackman, R. & Maselson, M. *Proc. natn. Acad. Sci. U.S.A.* **78**, 3775–3778 (1981).
- Mishina, M., *et al. EMBO J.* **1**, 1533–1538 (1982).
- Scott, R. W. & Tilghman, S. M. *Molec. cell. Biol.* **3**, 1295–1309 (1983).
- Karin, M. *et al. Nature* **308**, 513–519 (1984).
- Grosschedl, R. & Birnstiel, M. L. *Proc. natn. Acad. Sci. U.S.A.* **77**, 1432–1436 (1980).
- McKnight, S. L. & Kingsbury, R. *Science* **217**, 316–324 (1982).
- Grosschedl, R. & Birnstiel, M. L. *Proc. natn. Acad. Sci. U.S.A.* **79**, 297–301 (1982).
- Pelham, H. R. B. & Bienz, M. *EMBO J.* **1**, 1473–1477 (1982).
- Donahue, T. F., Farabaugh, P. T. & Fink, G. R. *Gene* **18**, 47–59 (1982).
- Maniatis, T., Fritsch, E. F. & Sambrook, J. *Molecular Cloning, A Laboratory Manual* (Cold Spring Harbor, New York, 1982).
- Maxam, A. & Gilbert, W. *Meth. Enzym.* **18**, 499–560 (1980).
- Sanger, F., Nicklen, S. & Coulson, A. R. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5463–5467 (1977).
- Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. *Biochemistry* **18**, 5294–5304 (1979).
- Lindberg, U. & Persson, T. *Eur. J. Biochem.* **31**, 246–254 (1972).
- Carmichael, G. G. & McMaster, G. R. *Meth. Enzym.* **65**, 380–391 (1980).
- Thomas, P. S. *Proc. natn. Acad. Sci. U.S.A.* **77**, 5201–5205 (1980).
- Suissa, M. *Analyt. Biochem.* **133**, 511–514 (1983).
- Berk, A. J. & Sharp, P. A. *Cell* **12**, 721–732 (1977).
- Weaver, R. F. & Weissman, C. *Nucleic Acids Res.* **7**, 1175–1193 (1979).
- Cashmore, A. R. in *Genetic Engineering of Plants, An Agricultural Perspective* (eds Kosuge, T., Meredith, C. P. & Hollander, A.) 29–38 (Plenum, New York, 1983).

First observation of ultra-high-energy γ rays from LMC X-4

R. J. Protheroe & R. W. Clay

Department of Physics, University of Adelaide, Adelaide, South Australia 5001, Australia

Ultra-high-energy γ rays ($>10^{15}$ eV) have recently been observed from Cygnus X-3 (refs 1, 2) and from Vela X-1 (ref. 3). The discovery that some neutron star binary X-ray sources are emitting ultra-high-energy (UHE) γ rays has led to considerable interest and speculation as to the nature of the cosmic particle accelerators responsible for the γ rays⁴⁻⁷ and to the notion that much of the galactic cosmic radiation above 10^{16} eV may originate from such objects⁷⁻⁹. Data from the University of Adelaide air-shower array at Buckland Park taken over a 3-year period have been analysed to search for evidence of UHE γ -ray emission from additional neutron-star binary X-ray sources having known orbital periods. We present here the result of this search and report the detection of UHE γ rays from LMC X-4 above 10^{16} eV with an integral flux of $(4.6 \pm 1.7) \times 10^{-11}$ photons $\text{m}^{-2} \text{s}^{-1}$. This is the first extragalactic object to be positively detected at these energies.

Observations of air showers were made continuously (except for maintenance) during 1979, 1980 and 1981 at Buckland Park (sea level, latitude 35°S). During this time, the array¹⁰ consisted of eleven 1-m^2 plastic scintillator detectors, five of which had fast-timing capability and were used for shower arrival direction analysis. Over the 3-year period some 1.3×10^5 air showers were recorded^{11,12} with primary energies $\geq 3 \times 10^{15}$ eV. Following our detection³ of UHE γ rays from Vela X-1, we examined the Buckland Park data to test for an excess of air showers from regions around the galactic plane and galactic centre¹³, and from the directions of known sources of γ rays at 100-MeV energies¹⁴. Here we describe our continued search for evidence of periodic emission of UHE γ rays from neutron-star binary X-ray sources. In addition to Vela X-1, there are 14 such objects in the southern celestial hemisphere which have known orbital periods. These objects are listed in Table 1 together with their type, estimated distance and orbital period. Of the neutron-star binary systems, 11 are massive and three are of low mass. Five (all massive systems) are in the Magellanic Clouds.

For each object, we examined the air showers that had apparent arrival directions within angle θ_c of the source direc-

tion. This 'resolution angle' depends on the declination¹⁴ and has been chosen so that 72% of any primary particles from the source direction would produce air showers that appear to arrive from within θ_c of the source direction. This procedure optimizes the signal-to-noise ratio. The observed number of events, n , is given in Table 1 together with the number of background cosmic-ray events expected, $\hat{B}$, based on air showers arriving from the same declination but from different right ascensions (the exposure ratio, α , is the ratio of the solid angle contained within θ_c of the source direction to that used in determining the background). All air showers with apparent arrival directions within θ_c were included in the analysis. Because the rate of detecting air showers at fixed shower electron number drops off very steeply with zenith angle¹⁴, the expected number of background events within θ_c of a source direction depends strongly on its declination (this is evident from the $\hat{B}$ values given in Table 1). Another consequence of this is that almost all the air showers analysed had zenith angles $<45^\circ$. This is in contrast to arrays comprising detectors sensitive to the (more penetrating) muon component, such as water Cerenkov detectors, which can readily detect showers to larger zenith angles.

In our observation³ of Vela X-1, and in the observation of Cyg X-3 by the Kiel group¹, it was assumed that the shower lateral-distribution age parameter, s , could be used to reduce the cosmic-ray background events by using only showers with large s . In both cases, the air showers containing an excess attributed to γ -ray initiated showers had only high s values. Furthermore, in observations¹⁵ of 'muon-depleted' showers, attributed to γ rays, the mean age parameter was found to be 1.4, whereas for 'ordinary' air showers it was 1.2. This seems to justify the use of an age cut. However, no age cut was used by the Haverah Park group² in their successful search for UHE γ -ray emission from Cyg X-3. The distribution in shower age depends on zenith angle and hence on declination. We find that the age distribution of cosmic-ray background events is quite broad if the declination of the suspected source differs greatly from the latitude of the air-shower array. The age distribution of γ -ray showers is also expected to be broad in this case. For such sources, the use of an age cut would not be helpful because of the greater overlap in age distributions of cosmic-ray and γ -ray showers. Of the sources listed in Table 1, only 1700-377 passes nearly overhead at Adelaide and only for this case was an age cut used to reduce the background of cosmic-ray events. For all the other sources, the source declination differs from the latitude of the air shower array by more than 17° and no age cut was used.

Table 1 Search for periodic emission of UHE γ rays from neutron star binaries

Source	Name	Type*	Distance† (kpc)	Period (day)	Ref.	θ_c (°)	α	$\hat{B}$	n	Y_n	$Y_{n,5\%}$	$Y_{n,1\%}$	E_{thr} (10^{15} eV)	n_y	F_y ($\text{m}^{-2} \text{s}^{-1}$)	L_y (erg s^{-1})	L_y/L_x
0115-737	SMC X-1	1	65	3.892387	20	2.7	0.044	25.1	23	5.07	5.53	5.85	15	10	4.4 (-11)	1.8 (+38)	0.3
0532-664	LMC X-4	1	50	1.40832	27	2.5	0.027	53.0	63	7.55	7.32	7.54	8	—§	—	—	—
0535-668	LMC Trans	1	50	16.6515	29	2.5	0.028	50.7	56	6.61	7.11	7.34	8	20	1.3 (-10)	1.6 (+38)	0.13
0538-641	LMC X-3	1	50	1.70480	30	2.4	0.025	63.6	73	7.40	7.59	7.80	7	26	1.6 (-10)	2.3 (+38)	0.8
0540-697	LMC X-1	1	50	3.909 4.039	31	2.6	0.033	39.8	44	6.45	6.67	6.92	10	18	9.9 (-11)	1.8 (+38)	0.9
0921-630	—	2	8	8.987	32	2.4	0.024	68.3	72	7.10	7.56	7.77	6	21	1.8 (-10)	4.4 (+36)	10.0
1119-603	Cen X-3	1	10	2.0871061	33	2.3	0.021	83.7	73	7.18	7.59	7.80	5	14	1.2 (-10)	6.1 (+36)	0.08
1223-624	GX301-2	1	2	41.50	34	2.4	0.023	72.6	69	7.41	7.48	7.70	6	16	1.2 (-10)	1.5 (+35)	0.015
1258-613	GX304-1	1	2.4	132.5	34	2.4	0.022	79.6	63	7.17	7.32	7.54	6	11	7.0 (-11)	1.8 (+35)	0.06
1516-569	Cir X-1	1	10	16.594	35	2.3	0.019	97.2	114	8.40	8.40	8.57	5	36	2.6 (-10)	1.2 (+37)	0.015
1538-522	—	1	7	3.730	36	2.2	0.016	118.0	109	7.76	8.32	8.50	4	18	1.6 (-10)	2.3 (+36)	0.6
1617-155	Sco X-1	2	0.7	0.787313	37	2.3	0.010	88.9	76	7.32	7.66	7.87	4	13	1.6 (-10)	1.4 (+34)	0.0005
1627-673	—	2	—	0.02883171 0.02884630	38	2.5	0.029	48.2	55	6.77	7.07	7.31	8	22	1.4 (-10)	—	—
1700-377 [‡]	—	1	1.7	3.41168	39	2.1	0.011	59.7	63	7.25	7.32	7.54	3	20	2.3 (-10)	9.0 (+34)	0.03

* 1, Massive system; 2, low-mass system.

† From ref. 23.

‡ γ -ray flux is above the effective energy threshold E_{thr} ; luminosity is per decade at 10^{16} eV assuming an E^{-2} differential photon spectrum at the source; X-ray luminosity is in the 2-11 keV range and is taken from ref. 23.

§ Positive detection, flux and luminosity given in text.

|| For this source, only showers with age parameters >1.3 were included in the analysis (see text).

When the observations were made, the Buckland Park array was sensitive to air showers with sea-level sizes $\geq 3 \times 10^5$ particles corresponding approximately to a primary energy of 3×10^{15} eV for nearly vertical showers. For large zenith angles, the threshold energies are higher. We have estimated the effective energy threshold of the array, E_{thr} , for showers incident from the declination of each object considered. This was based on the average atmospheric depth of the array as seen by showers from that declination and on an attenuation length¹² of 185 g cm^{-2} appropriate to showers of sizes about 10^6 particles. The effective threshold energies are given in Table 1.

To test for evidence of periodic emission of UHE γ rays in phase with the orbital motion of each binary system, the arrival time of each air shower was converted to an orbital phase, ϕ ($0 < \phi < 1$), after applying a heliocentric correction. Cosmic-ray events would be expected to have a uniform phase distribution and the hypothesis of uniformity was tested using the test statistic Y_n proposed by Protheroe¹⁶

$$Y_n = 2[n(n-1)]^{-1} \sum_{i=1}^{n-1} \sum_{j=i+1}^n (\Delta_{ij} + 1/n)^{-1} \quad (1)$$

where,

$$\Delta_{ij} = 0.5 - [|(\phi_i - \phi_j)| - 0.5] \quad (2)$$

This statistic was designed specifically for testing circular data for uniformity while being powerful against alternatives in the form of a uniform distribution plus a small narrow distribution at an arbitrary phase. The statistic Y_n representing the data is given in Table 1 together with the 5% and 1% critical value¹⁶ for each object. Y_n exceeded the 5% critical value for LMC X-4 and Cir X-1 but, because 14 objects were examined, only LMC X-4 for which Y_n exceeded the 1% critical value is considered to be significant.

For the 13 other objects, 95% upper limits¹⁷ to the expectation of the number of γ rays contributing air showers which arrive within θ_c are given in Table 1. Also given are 95% upper limits to the γ ray flux and luminosity. The latter takes into account a correction for attenuation by electron-photon cascading^{18,19} involving photon-photon pair production and inverse Compton interactions with photons of the 2.7-K microwave background assuming an E^{-2} photon spectrum at the source. In the case of galactic objects, the presence of the interstellar magnetic field rapidly removes energy from the cascade through synchrotron emission and so the photon-photon interactions were treated purely as an absorption process.

Figure 1 plots Y_n against period for the 63 events observed from within 2.5° of LMC X-4. Previous measurements of the orbital period²⁰⁻²², which are also indicated, are consistent with the range of periods for which Y_n is large.

The LMC X-4 observation may actually be more significant than suggested by the 1% significance level obtained using the Y_n statistic if the peak in the phase distribution is broader than the effective window width implied by equation (1), that is about n^{-1} of the period. The phase distribution of events from within 2.5° of the direction of LMC X-4 is plotted in Fig. 2. There is a significant excess of events between phases 0.90 and 0.95 where we observe 12 events when we expect 2.65 events. The probability, p , of this occurring by chance in a particular bin, obtained using the Poisson distribution, is 2.12×10^{-5} . The phase distribution is shown binned in 20 bins in Fig. 2, but was previously binned in 10 bins and this binning and rebinning must be taken into account when assigning a significance level for the observation. For LMC X-4 alone, the probability must then be increased to $1 - (1-p)^{(20+10)} = 6.36 \times 10^{-4}$ corresponding to a 3.2-standard-deviation observation. Because 14 sources were examined for UHE γ -ray emission, the probability that the observed phase distribution for LMC X-4 resulted from a chance fluctuation of cosmic-ray background events is $1 - (1-p)^{30 \times 14} = 0.009$. It is, therefore, probable (99.1% confidence) that the phase distribution is not the result of a background fluctuation.

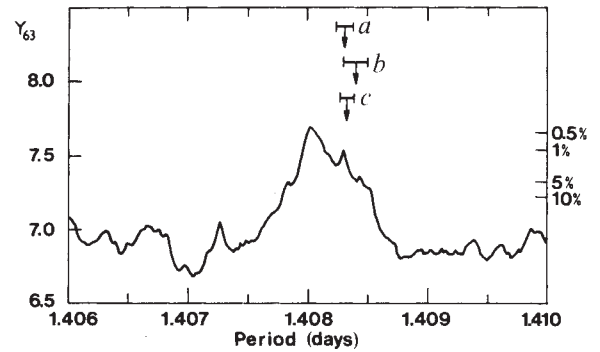


Fig. 1 The statistic Y_n derived from the phase distribution of the 63 air showers arriving from within 2.5° of LMC X-4 plotted as a function of period. Orbital periods derived by: a, Huchings *et al.*²¹; b, Lang *et al.*²²; and c, Kelley *et al.*²⁷ are indicated.

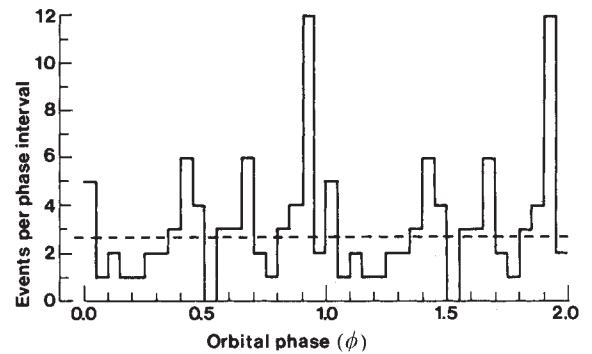


Fig. 2 Phase distribution of the 63 air showers observed within 2.5° of LMC X-4. The ephemeris of Kelley *et al.*²⁷ was used. The dashed line shows the expected phase distribution of the cosmic-ray background events.

We attribute the excess between phases 0.90 and 0.95, that is 9.4 ± 3.5 events, to γ rays. This corresponds to a time-averaged integral flux of $(4.6 \pm 1.7) \times 10^{-11} \text{ photons m}^{-2} \text{ s}^{-1}$ above 10^{16} eV. Assuming an E^{-2} photon spectrum at the source and taking account of electron-photon cascading in the 2.7-K microwave background, we obtain a luminosity of about $10^{38} \text{ erg s}^{-1}$ per decade which is of the same order of magnitude as that emitted at 2-11 keV energies²³. At ultra-high energies then, LMC X-4 has a luminosity ~ 20 times² that of Cyg X-3 and $\sim 5,000$ times³ that of Vela X-1, making it the most luminous object known at these energies. If the magnetic field between our Galaxy and the Large Magellanic Cloud exceeds $\sim 10^{-7} \text{ G}$, then the electron-photon cascade in the microwave background radiation field would be reduced by removing energy from it by synchrotron radiation¹⁸. In this case, to give the same flux of UHE γ rays the luminosity of LMC X-4 would need to be correspondingly higher by up to a factor of three.

Because LMC X-4 is in the Large Magellanic Cloud, some 50 kpc away, we would expect γ rays below $\sim 10^{16}$ eV to be severely attenuated in traversing the 2.7-K microwave background. This should be reflected in the energy spectrum of the γ rays. Unfortunately, the effective energy threshold of the array at the time of the observations was close to 10^{16} eV for showers incident from the declination of LMC X-4 and hence no test can be made with the current data for this effect. Recent extensions²⁴ to the Buckland Park array have increased its sensitivity to small air showers and future observations down to $\sim 10^{15}$ eV (for declination -67°) should reveal the presence of the absorption feature. Ideally, a new array at a more southerly latitude should be used to map out fully the absorption feature between below 10^{14} and 10^{16} eV. Such measurements have the potential to test the universality of the 2.7-K microwave background, measure the magnetic field between our Galaxy and the Large Magellanic Cloud, or to provide an independent measurement of the distance²⁵ to the Large Magellanic Cloud.

LMC X-4 is a massive binary system comprising an 07III-V or 08III-V star^{21,26} with a mass of $\sim 17 M_{\odot}$ together with a $\sim 1.6 M_{\odot}$ neutron star²⁷ in orbit with a 1.408-day period. The X-ray source has occasional flaring episodes during which $\sim 30\%$ of the X rays are pulsed²⁷ with a period of 13.5 s, presumably the spin period of the neutron star. The inclination²⁷ of the system is $\sim 66^{\circ}$ and the X-ray source is eclipsed between phase 0.92 and 0.08. If UHE protons are accelerated in the region of the compact object UHE γ rays could be produced by nuclear interactions in the atmosphere of the companion star. In this case, we would expect γ -ray emission at orbital phases ~ 0.92 and 0.08 when our line-of-sight to the neutron star just grazes the star's surface. The observation of UHE γ rays at a phase of 0.90-0.95 is consistent with this picture, although the absence of UHE emission at phases 0.05-0.10 is puzzling (a similar situation exists for Cyg X-3 and Vela X-1 where only one burst of UHE γ rays is observed per orbit). The X-ray source has high and low states associated with a 30.5-day period²² attributed to precession of an accretion disk. This precessing disk picture is supported by ultraviolet observations²⁸ which indicate an almost constant X-ray heating of the stellar atmosphere.

The star does not appear to fill its Roche lobe²¹ and seems to have a rather low stellar wind^{21,28}. Mass transfer may occur through a trailing accretion stream²¹ which may feed the accretion disk. Evidence for this comes from variable obscuration of the companion star²¹ between orbital phases 0.6 and 0.9 corresponding to matter trailing behind the neutron star by between 0.2 and 0.3 of an orbit. If this matter is of considerable extent above the orbital plane it could be the target material for interactions of UHE protons produced near the neutron star. Such a scenario would produce γ rays only at a phase of ~ 0.9 as observed.

We conclude that we have found evidence for UHE γ -ray emission by the LMC X-4 system modulated with the 1.408-day orbital period. The emission occurs when the neutron star is just entering its eclipse by the companion star and could result from nuclear interactions in the atmosphere of the companion by UHE particles produced near the neutron star. Alternatively, a trailing accretion stream could provide target material and explain the absence of UHE γ rays on leaving eclipse. Further observations in progress above 10^{15} and 10^{16} eV coupled with observations from a more southerly site at about 10^{14} eV could provide a test for the universality of the microwave background, be used as an indirect measurement of the magnetic field between our Galaxy and the Large Magellanic Cloud, or provide an independent measurement of the distance to the Large Magellanic Cloud.

We acknowledge the efforts of P. R. Gerhardy in obtaining the data-base analysed in the present work. Others particularly responsible for the development of the Buckland Park array have been J. R. Prescott, J. R. Patterson, A. G. Gregory, P. C. Crouch and D. F. Liebing. We thank D. F. Liebing and A. A. Watson for helpful comments and K. J. Orford and K. E. Turver for helpful discussions. R.J.P. thanks the Australian Government for a Queen Elizabeth II Fellowship. This work was supported in part by the Australian Research Grants Committee.

Received 22 November 1984; accepted 6 March 1985.

- Samorski, M. & Stamm, W. *Astrophys. J. Lett.* **268**, L17-L22 (1983).
- Lloyd-Evans, J. *et al. Nature* **305**, 784-787 (1983).
- Protheroe, R. J., Clay, R. W. & Gerhardy, P. R. *Astrophys. J. Lett.* **280**, L47-L50 (1984).
- Eichler, D. & Vestrand, W. T. *Nature* **307**, 613-614 (1984).
- Stephens, S. A. & Verma, R. P. *Nature* **308**, 828-830 (1984).
- Protheroe, R. J. *Nature* **310**, 296-298 (1984).
- Hillas, A. M. *Nature* **312**, 50-51 (1984).
- Wdowczyk, J. & Wolfendale, A. W. *Nature* **305**, 609-610 (1983).
- Wdowczyk, J. & Wolfendale, A. W. *Nature* **306**, 347-349 (1983).
- Crouch, P. C. *et al. Nucl. Instrum. Meth.* **179**, 467-476 (1981).
- Gerhardy, P. R. & Clay, R. W. *J. Phys. G: Nucl. Phys.* **9**, 1279-1287 (1983).
- Clay, R. W. & Gerhardy, P. R. *Aust. J. Phys.* **35**, 59-65 (1982).
- Clay, R. W., Protheroe, R. J. & Gerhardy, P. R. *Nature* **309**, 687-688 (1984).
- Protheroe, R. J. & Clay, R. W. *Proc. Astr. Soc. Aust.* **5**, 586-589 (1984).
- Gawin, J., Maze, R., Wdowczyk, J. & Zawadzki, A. *Can. J. Phys.* **46**, S75-S77 (1968).
- Protheroe, R. J. *Astr. Expr.* (submitted).
- Protheroe, R. J. *Astr. Expr.* **1**, 33-38 (1984).
- Wdowczyk, J. *et al. Proc. 12th int. Cosmic Ray Conf. (Hobart)* **3**, 965-967 (1971).

- Gould, R. J. *Astrophys. J. Lett.* **274**, L23-L25 (1983).
- van der Klis, M. *et al. Astr. Astrophys.* **106**, 339-344 (1982).
- Huchings, J. B., Crampton, D. & Cowley, A. P. *Astrophys. J.* **225**, 548-556 (1978).
- Lang, F. L. *et al. Astrophys. J. Lett.* **246**, L21-L25 (1981).
- Bradt, H. V. & McClintock, J. E. *A. Rev. Astr. Astrophys.* **21**, 13-66 (1983).
- Prescott, J. R. *et al. Proc. 18th int. Cosmic Ray Conf. Bangalore* **6**, 257-260 (1983).
- Cawley, M. F., Gibbs, K. & Weekes, T. C. *Proc. 18th int. Cosmic Ray Conf. Bangalore* **9**, 69-72 (1983).
- Chevalier, C. & Ilovaisky, S. A. *Astr. Astrophys.* **59**, L9-L12 (1977).
- Kelley, R. L. *et al. Astrophys. J.* **264**, 568-574 (1983).
- van der Klis, M. *et al. Astr. Astrophys.* **106**, 339-344 (1982).
- Skinner, G. K. *Space Sci. Rev.* **30**, 441-446 (1981).
- van der Klis, M., Tjemkes, S. & van Paradijs, J. *Astr. Astrophys.* **126**, 265-268 (1983).
- Huchings, J. B., Crampton, D. & Cowley, A. P. *Astrophys. J. Lett.* **275**, L43-L47 (1983).
- Cowley, A. P., Crampton, D. & Huchings, J. B. *Astrophys. J.* **256**, 605-611 (1982).
- Murakami, T. *et al. Astrophys. J.* **264**, 563-567 (1983).
- Priedhorsky, W. C. & Terrell, J. *Astrophys. J.* **273**, 709-715 (1983).
- Thomas, R. M. *et al. Mon. Not. R. astr. Soc.* **185**, 29P-32P (1978).
- Davison, P. J. N. *et al. Mon. Not. R. astr. Soc.* **181**, 73P-79P (1977).
- Gottlieb, E. W., Wright, E. L. & Liller, W. *Astrophys. J. Lett.* **195**, L33-L35 (1975).
- Middleditch, J. *et al. Astrophys. J.* **244**, 1001-1021 (1981).
- van Paradijs, J. *et al. Astr. Astrophys. Suppl.* **55**, 7-14 (1984).

Large losses of total ozone in Antarctica reveal seasonal ClO_x/NO_x interaction

J. C. Farman, B. G. Gardiner & J. D. Shanklin

British Antarctic Survey, Natural Environment Research Council, High Cross, Madingley Road, Cambridge CB3 0ET, UK

Recent attempts^{1,2} to consolidate assessments of the effect of human activities on stratospheric ozone (O_3) using one-dimensional models for 30°N have suggested that perturbations of total O_3 will remain small for at least the next decade. Results from such models are often accepted by default as global estimates³. The inadequacy of this approach is here made evident by observations that the spring values of total O_3 in Antarctica have now fallen considerably. The circulation in the lower stratosphere is apparently unchanged, and possible chemical causes must be considered. We suggest that the very low temperatures which prevail from midwinter until several weeks after the spring equinox make the Antarctic stratosphere uniquely sensitive to growth of inorganic chlorine, ClX , primarily by the effect of this growth on the NO_2/NO ratio. This, with the height distribution of UV irradiation peculiar to the polar stratosphere, could account for the O_3 losses observed.

Total O_3 has been measured at the British Antarctic Survey stations, Argentine Islands $65^{\circ}\text{S } 64^{\circ}\text{W}$ and Halley Bay $76^{\circ}\text{S } 27^{\circ}\text{W}$, since 1957. Figure 1a shows data from Halley Bay. The mean and extreme daily values from October 1957 to March 1973 and the supporting calibrations have been discussed elsewhere^{4,5}. The mean daily value for the four latest complete observing seasons (October 1980-March 1984) and the individual daily values for the current observing season are detailed in Fig. 1. The more recent data are provisional values. Very generous bounds for possible corrections would be ± 30 matm cm. There was a changeover of spectrophotometers at the station in January 1982; the replacement instrument had been calibrated against the UK Meteorological Office standard in June 1981. Thus, two spectrophotometers have shown October values of total O_3 to be much lower than March values, a feature entirely lacking in the 1957-73 data set. To interpret this difference as a seasonal instrumental effect would be inconsistent with the results of routine checks using standard lamps. Instrument temperatures (recorded for each observation) show that the March and October operating conditions were practically identical. Whatever the absolute error of the recent values may be, within the bounds quoted, the annual variation of total O_3 at Halley Bay has undergone a dramatic change.

Figure 1b shows data from Argentine Islands in a similar form, except that for clarity the extreme values for 1957-73 have been omitted. The values for 1980 to the present are provisional, the extreme error bounds again being ± 30 matm cm. The

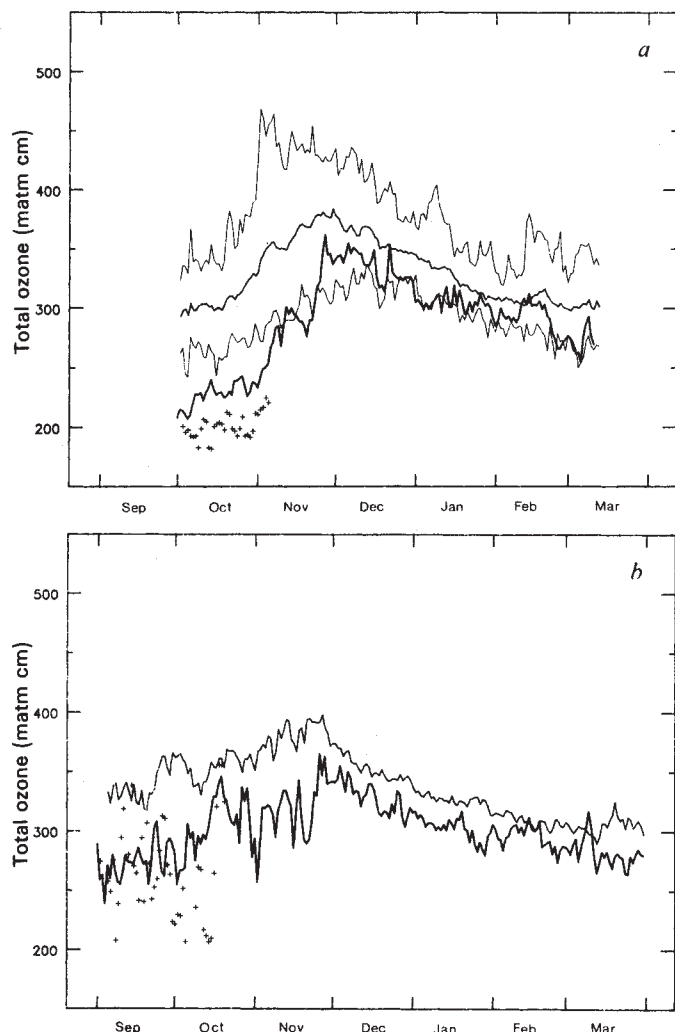


Fig. 1 Daily values of total O_3 . *a*, Halley Bay: thin lines, mean and extreme values for 16 seasons, 1957-73; thick line, mean values for four seasons, 1980-84; +, values for October 1984. Observing season: 1 October to 13 March. *b*, Argentine Islands: as for Halley Bay, but extreme values for 1957-73 omitted. Observing season: 1 September to 31 March.

changes are similar to those seen at Halley Bay, but are much smaller in magnitude.

Upper-air temperatures and winds are available for these stations from 1956. There are no indications of recent departures from established mean values sufficient to attribute the changes in total O_3 to changes in the circulation. The present-day atmosphere differs most prominently from that of previous decades in the higher concentrations of halocarbons. Figure 2*a* shows the monthly mean total O_3 in October at Halley Bay, for 1957-84, and Fig. 2*b* that in February, 1958-84. Tropospheric concentrations of the halocarbons F-11 ($CFCl_3$) and F-12 (CF_2Cl_2) in the Southern Hemisphere³ are also shown, plotted to give greatest emphasis to a possible relationship. Their growth, from which increase of stratospheric CIX is inferred, is not evidently dependent on season. The contrast between spring and autumn O_3 losses and the striking enhancement of spring loss at Halley Bay need to be explained. In Antarctica, the lower stratosphere is ~ 40 K colder in October than in February. The stratosphere over Halley Bay experiences a polar night and a polar day (many weeks of darkness, and of continuous photolysis, respectively); that over Argentine Islands does not. Figure 3 shows calculated amounts of NO_x in the polar night and the partitioning between the species⁶ Of these, only NO_3 and NO_2 are dissociated rapidly by visible light. The major reservoir, N_2O_5 , which only absorbs strongly below 280 nm, should be relatively long-lived. Daytime

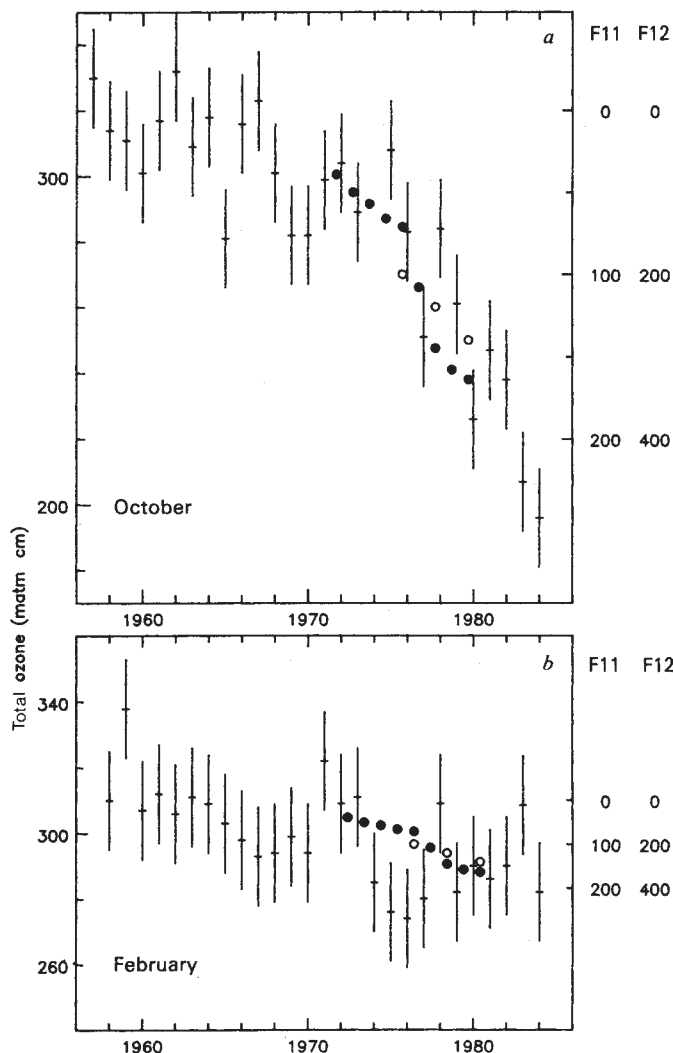


Fig. 2 Monthly means of total O_3 at Halley Bay, and Southern Hemisphere measurements of F-11 ($\bullet$, p.p.t.v. ($\text{parts per thousand by volume}$) $CFCl_3$) and F-12 ($\circ$, p.p.t.v. CF_2Cl_2). *a*, October, 1957-84. *b*, February, 1958-84. Note that F-11 and F-12 amounts increase down the figure.

levels of NO and NO_2 should be much less in early spring, following the polar night, than in autumn, following the polar day. Recent measurements⁷ support these inferences. The effect of these seasonal variations on the strongly interdependent ClO_x and NO_x cycles is examined below.

The O_3 loss rate resulting from NO_x and ClO_x may be written⁸

$$L = N + C = 2 k_2 [O] [NO_2] + 2 k_6 [O] [ClO] \quad (1)$$

L accounts for over 85% of O_3 destruction in the altitude range 20-40 km. At 40 km, N and C are roughly equal. Lower down, C decreases rapidly to 10% of L at 30 km, 3% at 20 km (refs 6, 8). Equation (1) is based on two steady-state approximations, (see Table 1*a* for the reactions involved)

$$\psi = \frac{[NO_2]}{[NO]} \sim \frac{k_1 [O_3] + k_4 [ClO]}{k_2 [O] + j_3} \quad (2)$$

and

$$\chi = \frac{[Cl]}{[ClO]} \sim \frac{k_6 [O] + k_4 [NO]}{k_5 [O_3]} \quad (3)$$

valid in daytime, with $[O]$ in steady state with $[O_3]$. Reaction (4) has a negative temperature coefficient, whereas reaction (1)

Table 1 Reaction list

a Governing ψ and χ (see text)		
$\text{NO} + \text{O}_3 \rightarrow \text{NO}_2 + \text{O}_2$	(1)	
$\text{NO}_2 + \text{O} \rightarrow \text{NO} + \text{O}_2$	(2)	
$\text{NO}_2 + h\nu \rightarrow \text{NO} + \text{O}$	(3)	
$\text{NO} + \text{ClO} \rightarrow \text{NO}_2 + \text{Cl}$	(4)	
$\text{Cl} + \text{O}_3 \rightarrow \text{ClO} + \text{O}_2$	(5)	
$\text{ClO} + \text{O} \rightarrow \text{Cl} + \text{O}_2$	(6)	
b Governing $[\text{Cl} + \text{ClO}]$		
$\text{HCl} + \text{OH} \rightarrow \text{Cl} + \text{H}_2\text{O}$	(7)	
$\text{ClONO}_2 + h\nu \rightarrow \text{ClO} + \text{NO}_2$	(8)	
$\text{HOCl} + h\nu \rightarrow \text{Cl} + \text{OH}$	(9)	
$\text{ClO} + \text{NO}_2 + \text{M} \rightarrow \text{ClONO}_2 + \text{M}$	(10)	
$\text{ClO} + \text{HO}_2 \rightarrow \text{HOCl} + \text{O}_2$	(11)	
$\text{Cl} + \text{CH}_4 \rightarrow \text{HCl} + \text{CH}_3$	(12)	
$\text{Cl} + \text{HO}_2 \rightarrow \text{HCl} + \text{O}_2$	(13)	
c		
$\text{HCl} + \text{ClONO}_2 \rightarrow \text{Cl}_2 + \text{HNO}_3$	(14)	

has large positive activation energy⁹, with the result that ψ is strongly dependent on $[\text{ClO}]$ at low temperature, as shown in Fig. 4. $[\text{ClO}]$ is not simply proportional to total ClX , because ClONO_2 formation (reaction (10)) intervenes. Throughout the stratosphere, $\chi \ll 1$, so that $[\text{ClO}] \sim [\text{Cl} + \text{ClO}]$. From a steady-state analysis of the reactions given in Table 1b,

$$[\text{Cl} + \text{ClO}] \sim \frac{k_7[\text{HCl}][\text{OH}] + j_8[\text{ClONO}_2] + j_9[\text{HOCl}]}{k_{10}[\text{NO}_2] + k_{11}[\text{HO}_2] + \chi(k_{12}[\text{CH}_4] + k_{13}[\text{HO}_2])} \quad (4)$$

Values of ψ , χ and $[\text{Cl} + \text{ClO}]$ obtained from equations (2), (3) and (4) are in good accord with full one-dimensional model results for late summer in Antarctica⁶. Neglecting seasonal effects other than those resulting from temperature and from variation of $[\text{NO} + \text{NO}_2]$, it is possible to solve simultaneously for $[\text{NO}_2]$ and $[\text{ClO}]$, and to derive L . Results are shown in Table 2 as relaxation times⁸, $[\text{O}_3]/L$, for various conditions. The spring values (lines 2, 3 and 4) are highly dependent on ClX amount (compare columns a and b), the autumn values (line 1) much less so. At Argentine Islands, the sensitivity to ClX growth should resemble that seen in line 2, attributable solely to low temperature. Lines 3 and 4 show the enhanced sensitivity possible at stations within the Antarctic Circle, such as Halley Bay, arising from slow release of $[\text{NO} + \text{NO}_2]$ following the polar night. It remains to be shown how stable O_3 budgets were achieved with the relaxation times for the lower chlorine level (Table 2, a).

Much O_3 destruction is driven by visible light, but production requires radiation below 242 nm. On the dates shown (Table 2), destruction persists for some 11 h, while, because of the long UV paths, production is weak (except around noon) at 29 km, and is virtually absent below that altitude. Line 1 of Table 2 then demands O_3 transport in autumn from the upper to the lower stratosphere, which is consistent with inferred thermally-driven lagrangian-mean circulations¹⁰. A mean vertical velocity of 45 m per day is in good accord with calculations of net diabatic cooling¹¹ and gives a realistic total O_3 decay rate in an otherwise conventional one-dimensional model⁶. The short

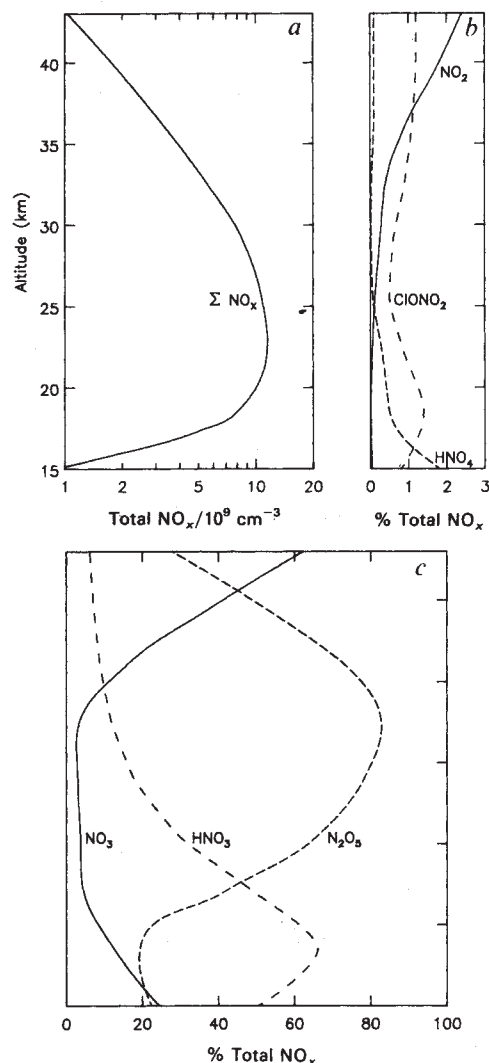


Fig. 3 NO_x during the polar night. a, Total NO_x cm^{-3} , from 15 to 43 km. b, NO_2 , ClONO_2 and HNO_3 as percentages of total NO_x . c, NO_3 , HNO_3 and N_2O_5 as percentages of total NO_x .

relaxation times in the lower stratosphere in autumn are tolerable, with adequate transport compensating for lack of O_3 production.

In early spring, on the other hand, wave activity scarcely penetrates the cold dense core of the Antarctic polar vortex and with very low temperatures the net diabatic cooling is very weak¹¹. Lagrangian transport in the vortex should then be almost negligible. (The virtual exclusion of Agung dust from the vortex supports this view⁵.) The final warming signals the end of this period of inactivity and is accompanied by large dynamically induced changes in O_3 distribution. However, before the warming, with low chlorine, total O_3 was in a state of near-neutral equilibrium, sustained primarily by the long relaxation times.

Table 2 Relaxation times in days, $[\text{O}_3]/L$, for maximum chlorine levels 1.5 p.p.b.v. (a) and 2.7 p.p.b.v. (b) (1980)

Date	Altitude (km)	22		25.5		29		32.5		36		39.5		43	
		Relative $\text{NO} + \text{NO}_2$													
		a	b	a	b	a	b	a	b	a	b	a	b	a	b
25 March	1	105	103	38	37	16	15	6.9	5.7	2.6	2.0	1.2	0.9	0.83	0.57
17 September	1	224	210	86	77	32	27	12.2	9.2	4.9	3.4	2.6	1.7	1.90	1.22
17 September	0.75	288	265	107	93	39	31	14.2	10.4	5.7	3.9	3.0	1.9	2.13	1.33
17 September	0.5	398	353	141	118	47	36	17.0	12.0	6.9	4.5	3.5	2.2	2.41	1.46

Noon values at 75.5°S, solar elevation 12.5°. Lower stratosphere at 230 K on 25 March; 190 K on 17 September. The altitudes shown apply to the summer temperature profile used in the model⁶.

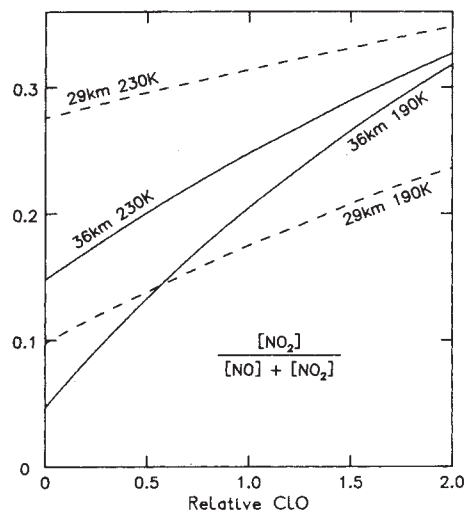


Fig. 4 $[\text{NO}_2]/[\text{NO} + \text{NO}_2]$ has the status of an efficiency factor for O_3 destruction by the NO_x cycle. In terms of the ratio ψ in the text, it is $\psi/(1 + \psi)$. The figure shows how this factor varies with $[\text{ClO}]$ at 190 K and at 230 K, at altitudes of 29 and 36 km. Values of $[\text{O}_3]$, $[\text{O}]$, $[\text{ClO}]$ and j_3 were taken from one-dimensional model results⁶ (maximum chlorine 2.7 p.p.b.v.) for noon, 25 March at 75.5° S, solar elevation 12.5°. The abscissa is $[\text{ClO}]$ relative to the model value. The rate-limiting reaction, (2) in Table 1, for the NO_x cycle has zero activation energy. Note how, nevertheless, O_3 was protected against destruction by NO_x at low temperatures in a stratosphere with small amounts of ClO , but is losing this protection as ClO grows.

With higher chlorine, relaxation times of the order seen in line 4, Table 2, entail more rapid O_3 losses. With negligible production below 29 km and only weak transport, large total O_3 perturbation is possible. The extreme effects could be highly localized, restricted to the period with diurnal photolysis between polar night and the earlier of either the onset of polar day or the final spring warming. At the pole $[\text{NO} + \text{NO}_2]$ rises continuously after the polar night, with the Sun. The final warming always begins over east Antarctica and spreads westwards across the pole. At Halley Bay the warming is typically some 14 days later than at the pole. Maximum O_3 depletion could be confined to the Atlantic half of the zone bordered roughly by latitudes 70 and 80° S.

Comparable effects should not be expected in the Northern Hemisphere, where the winter polar stratospheric vortex is less cold and less stable than its southern counterpart. The vortex is broken down, usually well before the end of the polar night, by major warmings. These are accompanied by large-scale subsidence and strong mixing, in the course of which peak O_3 values for the year are attained. Hence, sensitivity to ClO growth should be minimal if, as suggested above, this primarily results from O_3 destruction at low temperatures in regions where O_3 transport is weak.

We have shown how additional chlorine might enhance O_3 destruction in the cold spring Antarctic stratosphere. At this time of the year, the long slant paths for sunlight make reservoir species absorbing strongly only below 280 nm, such as N_2O_5 , ClONO_2 and HO_2NO_2 , relatively long-lived. The role of these reservoir species should be more readily demonstrated in Antarctica, particularly the way in which they hold the balance between the NO_x and ClO_x cycles. An intriguing feature could be the homogeneous reaction (Table 1c) between HCl and ClONO_2 . If this process has a rate constant as large as $10^{-16} \text{ cm}^3 \text{ s}^{-1}$ (ref. 2) and a negligible temperature coefficient, the reaction would go almost to completion in the polar night, leaving inorganic chlorine partitioned between HCl and Cl_2 , almost equally at 22 km for example. Photolysis of Cl_2 at near-visible wavelengths would provide a rapid source of $[\text{Cl} + \text{ClO}]$

at sunrise, not treated in equation (4). The polar-night boundary is, therefore, the natural testing ground for the theory of nonlinear response to chlorine^{1,2}. It might be asked whether a nonlinear response is already evident (Fig. 2a). An intensive programme of trace-species measurements on the polar-night boundary could add greatly to our understanding of stratospheric chemistry, and thereby improve considerably the prediction of effects on the ozone layer of future halocarbon releases.

We thank B. A. Thrush and R. J. Murgatroyd for helpful suggestions.

Received 24 December 1984; accepted 28 March 1985.

1. Cicerone, R. J., Walters, S. & Liu, S. C. *J. geophys. Res.* **88**, 3647–3661 (1983).
2. Prather, M. J., McElroy, M. B. & Wofsy, S. C. *Nature* **312**, 227–231 (1984).
3. *The Stratosphere 1981, Theory and Measurements* (WMO Global Ozone Research and Monitoring Project Rep. No. 11, 1981).
4. Farman, J. C. & Hamilton, R. A. *Br. Antarct. Surv. Sci. Rep.* No. 90 (1975).
5. Farman, J. C. *Phil. Trans. R. Soc. B279*, 261–271 (1977).
6. Farman, J. C., Murgatroyd, R. J., Silnickas, A. M. & Thrush, B. A. *Q. Jl R. met. Soc.* (submitted).
7. McKenzie, R. L. & Johnston, P. V. *Geophys. Res. Lett.* **11**, 73–75 (1984).
8. Johnston, H. S. & Podolske, J. *Rev. Geophys. Space Phys.* **16**, 491–519 (1978).
9. *Chemical Kinetics and Photochemical Data for Use in Stratospheric Modelling, Evaluation No. 6* (JPL Publ. 83–62, 1983).
10. Dunkerton, T. J. *J. atmos. Sci.* **35**, 2325–2333 (1978).
11. Dopplack, T. G. *J. atmos. Sci.* **29**, 1278–1294 (1972).

An Ordovician ophiolite in County Tyrone, Ireland

D. H. W. Hutton*, M. Aftalion† & A. N. Halliday†

* Department of Geological Sciences, University of Durham, Durham DH1 3LE, UK

† Scottish Universities Research and Reactor Centre, East Kilbride, Glasgow G75 0QU, UK

Ophiolites are regarded widely by geologists as remnants of old oceanic crust that has been obducted onto the continental margins during ocean or marginal basin closure and orogeny. Therefore, they provide evidence for the existence of old oceans and oceanic basins and are a key to the extension of plate tectonic modelling into pre-Mesozoic rock assemblages. In the Caledonide–Appalachian orogen, ophiolites are known although very few of these are dated well enough to provide accurate constraints on plate-tectonic kinematics. We report here the discovery of an ophiolitic sequence within the rocks of the Tyrone Igneous Complex in Northern Ireland. U/Pb dating combined with field evidence indicates that the Tyrone ophiolite was formed and then obducted onto the northern margins of the Iapetus Ocean close to 470 Myr BP (Arenig).

The Tyrone Igneous Complex (Fig. 1) occurs in an approximate along-strike continuation of the Midland Valley of Scotland; it is partly overlain by Upper Palaeozoic sediments within a shallow graben structure bounded by Dalradian rocks to the north and the Lower Palaeozoic of the Southern Uplands–Longford–Down zone to the south. The complex consists of three units (Fig. 1): a middle Ordovician (Llandeilo–Caradoc) volcanic sequence; a high metamorphic grade gneiss unit ‘the central inlier’; and a unit of gabbros and dolerites previously called ‘the basic plutonic complex’¹. Opinions on the age relationships and significance of the units have varied. Originally the basic plutonic complex and volcanics were grouped together as Llandeilo–Caradoc and regarded as being altogether younger and in tectonic contact with the central gneisses². Alternatively, the basic complex has been correlated with layered basic intrusions of Connemara and Aberdeenshire. This led to the view that both basic rocks and gneisses were older than and in unconformable contact beneath the Ordovician volcanic sequence³.

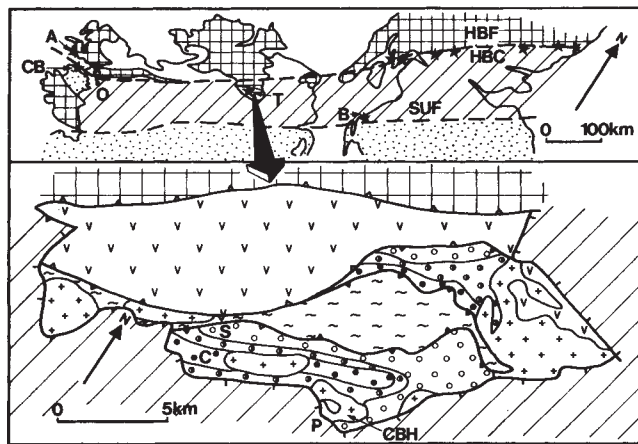


Fig. 1 *a*, Outline geological map of the Midland Valley zone in the British Isles (diagonal ruling). Southern Uplands zone (stipple). Dalradian and older (cross hatch). HBF, Highland Boundary Fault; SUF, Southern Uplands Fault; Caledonian ophiolites: T, Tyrone; B, Ballantrae; HBC, Highland Border Complex; A, Achill Island; CB, Clew Bay; O, Ox Mountains. *b*, Outline geological map of the Tyrone Igneous Complex. Dalradian (cross hatch); Upper Palaeozoic-Tertiary cover (diagonal ruling); Llandeilo-Caradoc volcanics (V); granite (+); central inlier gneisses (~); ophiolite sheeted dykes (●); ophiolite transition (⊙); ophiolite gabbros (○); tectonic contacts (barbed lines); unconformities (toothed lines); C, Carrickmore; P, Pomeroy; CBH, Craigballyhark pluton; S, the Scalp.

Reconnaissance remapping and reassessment of the 'basic plutonic complex' (D.H.W.H.) indicates that this is part of an ophiolite 35 × 10 km in area. The lowermost exposed part of this is essentially gabbroic with variably developed cumulate layering⁴ as well as leucogabbros and isotropic and pegmatoid gabbros. Overlying this and previously unrecognized as such is a sheeted dyke complex. This is most clearly seen in Carrickmore Quarry [609 721] (Irish National Grid), which exposes 100% dolerite made up of parallel dykes averaging 1 m in thickness. These intrude each other to produce both two-sided chills and, more commonly, the classic one-sided chilled margins typical of sheeted dyke complexes. Elsewhere in the Carrickmore area and along strike for 18 km to the north-east and in another slice of the ophiolite to the north, this dolerite is found and is composed entirely of dykes cutting dykes. The dykes in the complex have general north-east south-west trends and are vertical-to-moderately south dipping. In the lower parts of the sheeted complex the dykes are often deformed parallel to their margins. Overlying the dolerites and so far recorded in only two localities (Carrickmore², and Craigballyhark [732 755]) are a few metres of spilitized basaltic pillow lavas with thin chert screens and fragments.

Separating the sheeted dyke complex from the gabbros is a complicated transition zone. This contains early sheeted-dyke dolerites, often strongly deformed, which are cut and intruded by isotropic and pegmatitic gabbros as irregular bodies and veins. This zone is probably the result of repeated intrusion of the gabbro magma chamber in the vicinity of the spreading

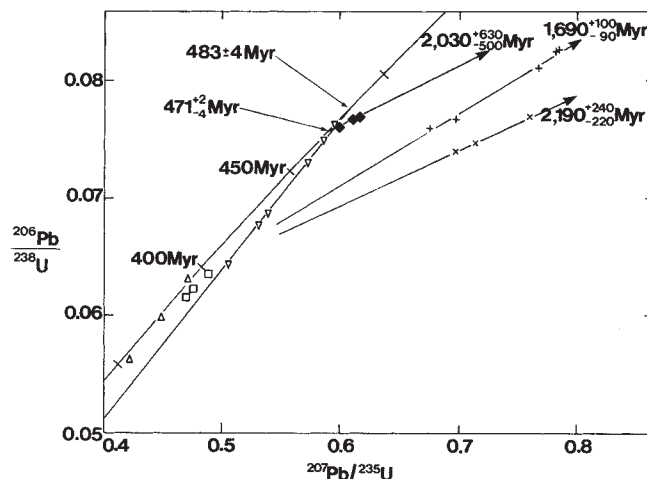


Fig. 2 U/Pb concordia diagram showing data for zircons from the Craigballyhark tonalite and, for comparison, other local rock types, namely, the Ballantrae complex¹² (the nearest other ophiolite), late Caledonian (~400 Myr BP) granites (Distinkhorn²⁸ and Loch Doon¹⁰), a greywacke from the Southern Uplands of Scotland¹⁰ and a granulite facies xenolith from the Midland Valley of Scotland¹¹. ♦, Craigballyhark tonalite R.C.2005; ▽, Ballantrae trondhjemite R.C.1502; □, Loch Doon granite R.C.1812; △, Distinkhorn granite R.C.965; +, S. Uplands greywacke R.C.1813; ×, Granulite facies metasedimentary xenolith, Partan Craig R.C.1861.

centre into the overlying sheeted-dyke complex. The deformed dolerite dykes probably represent ductile extension fault development sub-parallel to the dykes, a consequence of newly formed oceanic lithosphere subsiding away from the ridge crest during cooling and crystallization⁵.

Within the lower part of this zone and in the gabbro unit, at, for example, the Scalp⁴ [636 745], there are common small intrusive sheets, bodies and net vein systems of tonalite, quartz diorite and trondjemite. These have complex time relationships with the basic rocks and the deformation and are interpreted as silicic differentiates from the developing ocean crust. Apart from older records of small serpentine occurrences in Tyrone and the identification in the present study of clinopyroxenite pegmatites (thought to be typical of the gabbro-peridotite contact in ophiolites⁶), there are no ultrabasic rocks present. This is probably the result of thrusting, because all the external contacts of the ophiolite are tectonic.

A feature of the entire basic complex that previous models could not easily explain is the widespread development of moderate-to-high-grade metamorphism. The gabbros and dolerites are irregularly uranitized to epidote amphibolite metamorphic assemblages without, in general, any accompanying deformation. This is typical of the metamorphism of many ophiolites and is probably the result of metasomatic metamorphism by circulating hot brines in the oceanic environment⁷.

The sequence described above, which occurs in two tectonic slices separated by the central inlier, is clearly part of an ophiolite⁸. The bottom of the ophiolite has been removed

Table 1 U/Pb isotope data for zircons from Craigballyhark pluton, Tyrone

Size fraction (μm)*	Pb (p.p.m.)	U (p.p.m.)	²⁰⁶ Pb/ ²⁰⁴ Pb		Atom. % ²⁰⁷ Pb	²⁰⁸ Pb	Atomic ratios			Apparent age (Myr BP)		
			²⁰⁶ Pb	²⁰⁴ Pb			²⁰⁷ Pb/ ²⁰⁶ Pb	²⁰⁷ Pb/ ²³⁵ U	²⁰⁶ Pb/ ²³⁸ U	²⁰⁷ Pb/ ²⁰⁶ Pb	²⁰⁷ Pb/ ²³⁵ U	²⁰⁶ Pb/ ²³⁸ U
-85	12.4	138	2,429	74.38	4.30	21.42	0.057939	0.61533	0.077020	528	487	478
-85+70	14.7	151	2,403	68.25	3.94	27.81	0.057699	0.61236	0.076967	518	485	478
-53	14.9	161	2,260	70.77	4.03	25.20	0.056945	0.59819	0.076183	489	476	474

* All size fractions analysed were non-magnetic at a side inclination of 1°. Uncertainty in ²⁰⁷Pb/²³⁵U and ²⁰⁶Pb/²³⁸U estimated at 0.3% and 0.2% (1σ) respectively. Regression of data using the method of York²⁷ yields upper intercept of 2,030⁺⁶³⁰₋₅₀₀ Myr BP and lower intercept of 471⁺²₋₄ Myr BP (SUMS = 0.52). All isotope measurements were made using a VG Micromass MM308 mass spectrometer.

tectonically and much of the uppermost (basalt-sediment) part has been removed by tectonism or erosion or both. The Tyrone ophiolite is not equivalent to the Connemara and Aberdeenshire layered basic complexes by virtue of its structure and its non-intrusive nature.

The Tyrone ophiolite is cut by a number of small granitic plutons, many of which are biotite and hornblende granites and granodiorites and may be Devonian in age². The Craigballyharky pluton however, which occurs near the village of Pomeroy [732 755], is a tonalite quartz-diorite granodiorite body⁹ that intrudes the ophiolite. The marginal quartz diorite of the Craigballyharky intrusion has a hybrid igneous character that has been interpreted as the result of interaction and mixing between the ophiolitic basic country rocks and the intruding central tonalite magma⁹. To achieve such mixing, the basic rocks themselves must have been at quite a high temperature. As there is no evidence in the area for a separate post-obduction pre-granite heating event of the ophiolite, this high temperature must have been a relic of the ophiolite ocean floor metamorphism. Thus the age of emplacement of the tonalite must approximate the age of the ophiolite itself or be slightly later than it.

In Table 1 we report U/Pb isotope data for three zircon size fractions from a sample of the main tonalite unit at Craigballyharky. Techniques are described elsewhere¹⁰ and are summarized in the table footnote. The results are moderately discordant and when plotted on a conventional concordia diagram (Fig. 2) define a discordia line with a lower intercept of 471^{+2}_{-4} Myr BP and an upper intercept of $2,030^{+630}_{-500}$ Myr BP (2σ).

We interpret the lower intercept as the age of intrusion of the Craigballyharky tonalite. This, from the foregoing arguments, places close constraints on the age of the ophiolite itself, which was formed at or just before 471^{+2}_{-4} Myr BP (Arenig). The ophiolite is therefore older and quite distinct from the Llandeilo-Caradoc (~455 Myr BP) volcanic sequence with which it has been correlated in the past.

The upper intercept indicates that the source of the magma and/or the material assimilated by the magma contained zircons of early Proterozoic age. This result is very similar to that obtained for zircons in Ordovician greywackes from the Southern Uplands of Scotland¹⁰ and also from a metasedimentary granulite-facies xenolith from a vent in the Midland Valley of Scotland¹¹. The U concentrations of the Craigballyharky zircons (Table 1) are among the lowest recorded from rocks of the British Isles and are very similar to those of zircons from the Ballantrae trondjemite¹² and to zircons from, again, the Midland Valley granulite-facies xenolith¹¹. Such low concentrations are expected from granitic regimes that have been melted from basic oceanic crust or from granulite facies continental crust. A plausible explanation to these data, therefore, is that the tonalite represents a mixture of both a mafic component from a relatively young ocean crust and a felsic component from old (Proterozoic) continental crust. We suggest that this crust was part of the northern continental margin to the Iapetus Ocean at this time. Thus, the tonalite rose through the margin and cut the ophiolite very soon after it had been obducted. Hence ophiolite formation and obduction occurred close together in time at 471^{+2}_{-4} Myr BP (Arenig).

The age of the Tyrone ophiolite compares well with the age of the Bay of Islands complex ($475\text{--}515$ Myr BP)^{13,14}, Ballantrae (~480 Myr BP)^{12,15}, Shetland ($465\text{--}479$ Myr BP)¹⁶ and Thetford in Quebec (~490 Myr BP)¹⁷. The Tyrone data provides an important link between the Newfoundland and Scottish ophiolites and confirms that, during the late Tremadoc-Arenig, ophiolite formation and obduction was probably widespread along the Caledonide-Appalachian system. In the British Isles it is perhaps significant that the Caledonian ophiolites north of the Iapetus suture all lie in the Midland Valley of Scotland and its extension into Ireland. Thus, in Scotland the ophiolitic fragments of early Arenig age or older that occur in the Highland Border complex¹⁸ may be comparable with at least part of the Ballantrae ophiolitic sequence seen further to the south^{12,15} (Fig. 1). In Ireland, Tyrone, the largest of these ophiolites, has

obvious similarities in both age and setting to Ballantrae. Further west in Ireland the undated Clew Bay ophiolitic melange¹⁹ and the Achill Island serpentinitic melange²⁰ are disrupted Caledonian ophiolites that may be compared with the Highland Border rocks. Also, nearby in the Ox Mountains, small scraps of Caledonian serpentinite are recorded²¹. One view of these ophiolite occurrences in the Midland Valley is that a closed ocean or oceanic basin is concealed at the site of the Highland Boundary or Southern Uplands fault^{19,22}. An alternative view, presented here, is that the Midland Valley basement is part of the Proterozoic North American continental margin to Iapetus, onto which ophiolites were obducted from the south during the Ordovician. The Highland Boundary and Southern Upland fault have been identified recently^{23,24} as long lived Palaeozoic transcurrent faults. We suggest that the Midland Valley became detached from the North American continent along these faults by sinistral movements, probably culminating in the early Devonian^{25,26}, which displaced this allochthonous terrane north-eastwards into its present position.

Received 28 January; accepted 13 March 1985.

1. Wilson, H. E. *Regional Geology of Northern Ireland* (HMSO, Belfast, 1972).
2. Hartley, J. J. *Proc. R. Ir. Acad.* **B41**, 218-285 (1933).
3. Cobbing, J., Manning, P. I. & Griffith, P. E. *Nature* **206**, 1132-1135 (1965).
4. Cobbing, J. *Bull. geol. surv. Gt Br.* **30**, 88-99 (1969).
5. Rosencrantz, E. *Can. J. Earth Sci.* **20**, 787-801 (1983).
6. Dewey, J. F. & Bird, J. M. *J. geophys. Res.* **76**, 3179-3206 (1971).
7. Moody, J. B. *Can. Miner.* **17**, 871-887 (1979).
8. Anon. *Geotimes* **17**, 24-25 (1972).
9. Angus, N. S. *Proc. R. Ir. Acad.* **B77**, 181-199 (1977).
10. Halliday, A. N., Aftalion, M., van Breemen, O. & Jocelyn, J. *Geol. Soc. Lond. spec. Publ.* **8**, 653-661 (1979).
11. Halliday, A. N., Aftalion, M., Upton, B. G. J., Aspen, P. & Jocelyn, J. *Trans. R. Soc. Edinb. Earth. Sci.* **75**, 71-74 (1984).
12. Bluck, B. J., Halliday, A. N., Aftalion, M. & Macintyre, R. M. *Geology* **8**, 492-495 (1980).
13. Archibald, D. A. & Farrar, E. *Can. J. Earth. Sci.* **12**, 520-529 (1976).
14. Jacobsen, S. B. & Wasserburg, G. J. *J. geophys. Res.* **84**, 7429-7445 (1979).
15. Thirlwall, M. F. & Bluck, B. J. *Geol. Soc. Lond. spec. Publ.* **13**, 215-230 (1984).
16. Prichard, H. M. & Spray, J. G. *J. geol. Soc. Lond.* (in the press).
17. Clague, D., Rubin, J. & Brackett, R. *Can. J. Earth. Sci.* **18**, 469-486 (1981).
18. Curry, G. B. *et al. Trans. R. Soc. Edinb. Earth. Sci.* **75**, (in the press).
19. Ryan, P. D., Sawal, V. K. & Rowlands, A. S. *Nature* **301**, 50-52 (1982).
20. Kennedy, M. J. *J. Earth. Sci. R. Dublin Soc.* **3**, 117-126 (1980).
21. Long, C. B. & Max, M. D. *J. geol. Soc. Lond.* **133**, 413-432 (1977).
22. Max, M. D., Ryan, P. D. & Inamdar, D. D. *Tectonics* **2**, 431-451 (1983).
23. Bluck, B. J. *Trans. R. Soc. Edinb. Earth. Sci.* **75** (in the press).
24. Elders, C. F. *Abstr. Tectonic Studies Group Meet.* (Geological Society, London, 1984).
25. Soper, N. J. & Hutton, D. H. W. *Tectonics* **3**, 781-794 (1984).
26. Dewey, J. F. & Shackleton, R. M. *Nature* **312**, 115-121 (1984).
27. York, D. *Earth planet. Sci. Lett.* **5**, 320-324 (1969).
28. Pidgeon, R. T. & Aftalion, M. *Geol. J. spec. Iss.* **10**, 183-248 (1978).

Seismic sequences in Greece interpreted in terms of barrier model

G. F. Karakaisis, B. G. Karacostas, E. E. Papadimitriou, E. M. Scordilis & B. C. Papazachos

Geophysical Laboratory, University of Thessaloniki, Thessaloniki, Greece

Between 1978 and 1981, five shallow main shocks occurred in Greece and the surrounding region. Here we consider the space-time distribution of the foreshocks and the aftershocks of these earthquakes by interpreting the migration characteristics of the epicentres in terms of the barrier model¹. This model suggests that segmented ruptures can occur in a seismic fault but that the rupture front may be stopped by a barrier, or may propagate over the whole fault plane leaving the barrier unbroken. These unbroken barriers result in aftershocks due to the concentration of stress there, and cause migration of major events along a plate boundary. This migration may be an important factor for the prediction of earthquakes.

According to the barrier model, segmented ruptures could occur in a seismic fault, that is, slip could occur in cracks during the fault rupture process, while the region between cracks remains unbroken after the rupture. A rupture front may be

stopped by a barrier, but elastic waves generated by the slip can break the fault plane ahead of the barrier. Then, the rupture can propagate over the whole fault plane to leave the barriers unbroken. The residual stress over the fault plane immediately following the rupture is not uniform. Consequently, the stress will concentrate at the unbroken barriers² and cause aftershocks. Barriers also cause migration of major events along a plate boundary, such as the westward migration of great earthquakes³ on the Anatolian fault which took place between 1939 and 1944.

During 1978–81, five shallow main shocks with surface wave magnitudes ≥ 6.5 occurred in Greece and the surrounding area. Table 1 lists the focal coordinates (ϕ , λ , h), the surface wave magnitude (M_s), number of observations (N), mean error of the origin time (r.m.s.), and mean error in the location (ERH, km) for the main shocks.

To determine accurate locations of the epicentre and the focal depth, we used the HYPO 71 computer program⁸, adopting a two-layer crustal model above a half space⁹ with the following thicknesses (d_i) of the layers, and velocities (V_i); $d_1 = 19.0$ km, $V_1 = 6.0$ km s⁻¹, $d_2 = 12.0$ km, $V_2 = 6.6$ km s⁻¹. The P-wave velocity in the half space has been taken as 7.9 km s⁻¹. The accuracy in the determination of the focal parameters has been improved by using time delay corrections¹⁰ on the arrival times at the seismological stations in Greece and neighbouring countries. The average ERH for all the shocks of the five seismic sequences is satisfactory for our purpose, being 2.0 ± 0.9 km.

Figure 1 is a map of Greece and of the surrounding area showing the strikes of the faults producing the five seismic sequences. The main source parameters of these earthquakes are known¹¹. To investigate the space-time distribution of the epicentres of the five seismic sequences, the position of the epicentre projection on the strike direction of the fault plane, which is the largest dimension of the aftershock area, is plotted against time for each sequence. The distances from the epicentre, x , are always measured from a point, A, which is located at one of the two ends of the fault's strike, because the epicentre migration takes place parallel to this direction.

Figure 2 shows the space-time diagrams of the five seismic sequences. In three of these, the main shock was preceded by smaller shocks. In the Thessaloniki 1978 seismic sequence (Fig. 2a), these shocks migrated towards the main shock epicentre, that is, from east to west, while in Montenegro 1979 and Magnesia 1980 seismic sequences (Fig. 2b, c) these shocks are very closely related, in space and time, with the main shock. This clearly shows that these earthquakes are real foreshocks of the three main shocks.

In all five cases, after the main shock, the aftershock activity expanded from the focus of the main shock to one (Fig. 2a, c, d) or both ends (Fig. 2b, e) of the fault, reaching these ends after 2 h, 24 min, 7 h, 15 min and 30 min respectively.

Figure 2 shows that parts of the faults do not have aftershocks. For example the central parts of the faults which produced the Monte-Negro 1979 (Fig. 2b), the Magnesia 1980 (Fig. 2c), the Alkyonides Gulf 1981 (Fig. 2d) and the Northern Aegean 1981 (Fig. 2e) seismic sequences. These are the parts of the faults which presumably slipped smoothly during the main shocks. The dashed circles in Fig. 2d denote shocks related to an antithetic fault parallel to the main fault.

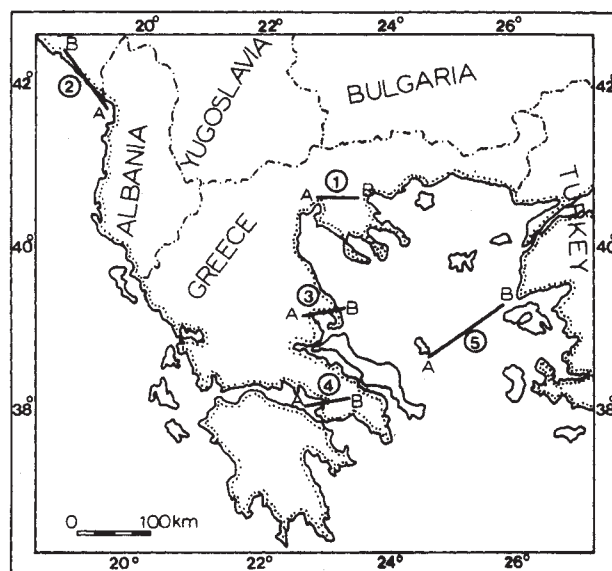


Fig. 1 Index map indicating the strikes, AB, of the main faults of the five seismic sequences. Migration of these shocks in each sequence was investigated along these strikes. Positions 1–5 correspond to a–e of Fig. 2.

The aseismic slip induced high tectonic stress at one or both ends of the fault where the clustering of aftershocks is observed. The clustering probably indicates the sites of the barriers where high stress was induced following the main shocks.

After a time interval, which varies from sequence to sequence, the stress reached the barrier's ultimate strength, breaking the strongest barrier and producing the largest aftershock in each seismic sequence.

Three out of five main shocks with $M_s \geq 6.5$ were preceded by recorded foreshocks. These foreshocks started 5 to 42 days before the main shocks and were either very close to the epicentres of the main shocks or their epicentres migrated to the epicentres of the main shocks. Detailed studies of foreshocks could, therefore, contribute to the prediction of the main shocks.

In three of the seismic sequences, the focus of the main shock was located in the middle section of the fault; in the other two cases, it was located near one end of the fault. Thus, the focus of the main shock can be anywhere in the fault.

In all five cases, the aftershock activity expanded to one or to both ends of the fault after the main shock and reached these ends within one day. In all five cases, the largest aftershocks occurred near the one end of the fault within a time period which varied from sequence to sequence between 15 min and 38 days after the main shock. This observation shows that a continuous monitoring of the aftershock activity can lead to the prediction of the epicentre of the largest aftershocks which sometimes produce damage comparable with that of the main shock.

The barrier model gives a general explanation of the occurrence of the shocks of the seismic sequences, but detailed knowl-

Table 1 Data for the main shocks of the five sequences

No.	Date	Origin time	Location		h (km)	M_s	N	r.m.s. (s)	ERH (km)	Ref.
			ϕ (°N)	λ (°E)						
1	20 June 1978	20:03:23	40.73	23.25	8	6.5	385	—	—	4
2	15 April 1979	06:19:41	41.93	18.99	1	7.1	33	0.46	1.4	5
3	9 July 1980	02:11:57	39.28	23.11	9	6.5	17	0.50	2.4	6
4	24 February 1981	20:53:37	38.16	22.88	10	6.7	36	0.39	1.5	7
5	19 December 1981	14:10:52	39.08	25.24	3	7.3	36	0.43	1.3	*

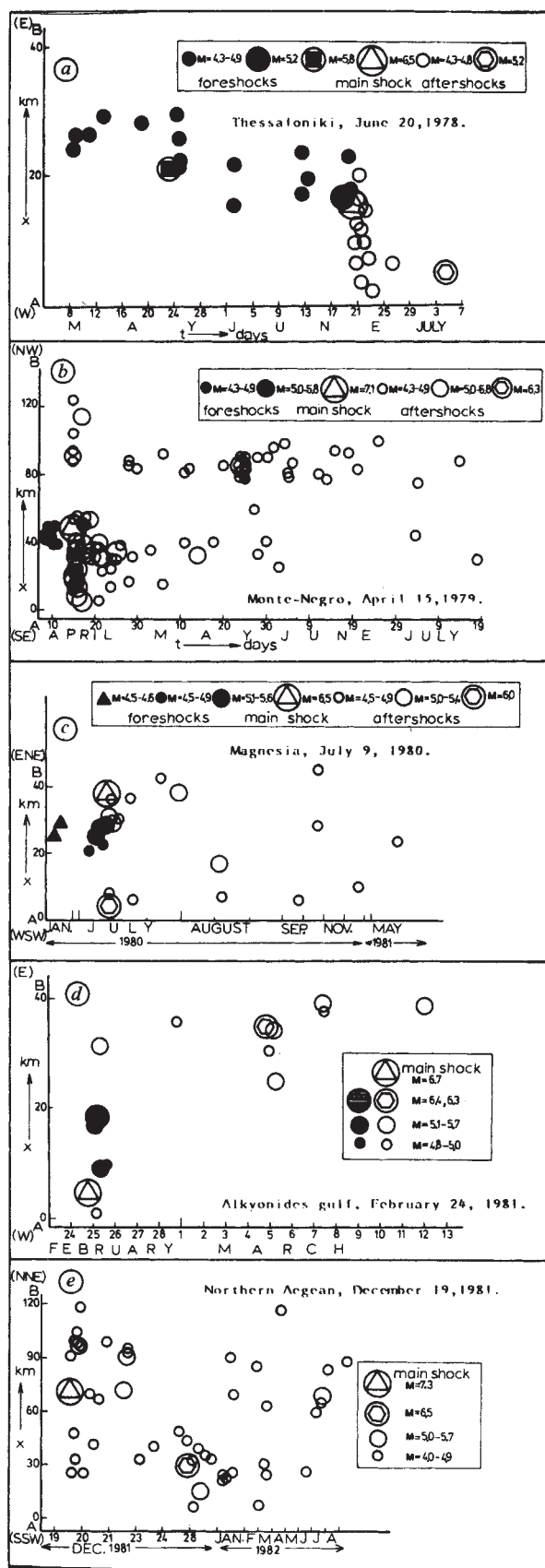


Fig. 2 Space-time distribution of the five seismic sequences. a, Thessaloniki, 1978; b, Montenegro, 1979; c, Magnesia (central Greece), 1980; d, Alkyonides Gulf, 1981; e, northern Aegean, 1981.

edge of the space-time distribution of shocks would improve this model.

Received 29 November 1984; accepted 8 March 1985.

1. Aki, K., Bouchon, M., Chouet, B. & Das, S. *Ann. Geofis.*, **30**, 341-368 (1977).
2. Aki, K. *Bull. seism. Soc. Am.* **72**, S29-S41 (1982).
3. Papageorgiou, A. S. thesis, Massachusetts Institute of Technology (1981).
4. Papazachos, B. C., Tsapanos, T. M. & Panagiotopoulos, D. G. *Nature* **296**, 232-235 (1982).
5. Karakaisis, G. F., Karacostas, B. G., Papadimitriou, E. E. & Papazachos, B. C. *Pageophysics* (in the press).
6. Papazachos, B. C., Mountrakis, D. M., Dimapoulos, G. Ch., Panagiotopoulos, D. G. & Tsapanos, T. M. *Geophys. J. R. astr. Soc.* **75**, 155-168 (1983).
7. Papazachos, B. C., Comninakis, P. E., Papadimitriou, E. E. & Scordilis, E. M. *Ann. Geophys.* **2**, 537-544 (1984).
8. Lee, W. H. K. & Lahr, J. C. *U.S. geol. Surv.* **75**, 311 (1975).
9. Panagiotopoulos, D. G. thesis, Univ. Thessaloniki (1984).
10. Panagiotopoulos, D. G. & Papazachos, B. C. *Geophys. J. R. astr. Soc.* **80**, 165-176 (1985).
11. Kiratzi, A. A., Karakaisis, G. F., Papadimitriou, E. E. & Papazachos, B. C. *Pageophysics* (in the press).

The origin of authigenic ankerite from the Ninian Field, UK North Sea

J. D. Kantorowicz*

Department of Geology, University of Hull, Hull HU6 7RX, UK

The carbonate minerals ankerite and ferroan dolomite are widely reported in studies of concretions^{1,2} and of sandstone hydrocarbon reservoirs³⁻⁸. These minerals are often thought to have formed relatively late in the sandstones' diagenetic history and in the Gulf Coast their occurrence has been linked to clay mineral diagenesis in the surrounding shales⁹. I describe here authigenic ankerite from the Ninian Field¹⁰. Ankerite formation was probably synchronous with hydrocarbon emplacement and is related to clay mineral diagenesis in the hydrocarbon source rocks of the Viking Graben. It occurs throughout the reservoir sandstones but is particularly abundant below the oil-water contact. This distribution reflects hydrocarbon emplacement arresting diagenesis at progressively deeper levels of the reservoir. The resulting preferential cementation may adversely affect transmissibility between the aquifer and the oil column and consequently could reduce hydrocarbon recovery.

Two forms of authigenic ankerite are observed in cores from Wells 3/3-1, -2 and -3 from the Ninian Field in the northern North Sea. The reservoir comprises Middle Jurassic Brent Group sandstones¹⁰ and is characterized by a complex diagenetic mineralogy including clay minerals, quartz and feldspar overgrowths, siderite, pyrite, calcite and barytes¹¹. The two forms of ankerite occur irrespective of depositional facies and enclose and replace the earlier formed authigenic minerals. The first form is concretionary and occurs in finer-grained sediments. It comprises large poikiloblastic crystals several millimetres in length which totally fill pore space and constitute 23-50% of the bulk rock volume. The second form is discrete pore-filling rhombs, 20-40 μm in length which form up to 1% of the bulk rock volume above the oil-water contact, but up to 5% below.

Carbon and oxygen stable isotope ratios of ankerite samples are compiled in Table 1. Carbon isotope ratios can provide information on the origin of the bicarbonate in carbonate minerals¹. Carbonates precipitating from seawater have a $\delta^{13}\text{C}$ of 0‰ PDB. Also, carbonates incorporating bicarbonate from specific bacterial degradation reactions have specific $\delta^{13}\text{C}$ compositions of -20 to -25 or +15‰ (ref. 1) or intermediate values if bicarbonates are liberated by more than one mechanism. The carbon isotope compositions of these ankerite samples ($\delta^{13}\text{C}$ -7.1 to -13.6) are not indicative of a specific source of bicarbonate. These values could result from precipitation in pore waters supplied by two sources of bicarbonate. Alternatively, this composition could reflect the dissolution, equilibration and reprecipitation, of *in situ* carbonate cements of one

* Present address: Koninklijke/Shell Exploratie en Productie Laboratorium, Volmerlaan 6, Rijswijk, The Netherlands.

isotopic composition with CO_2 produced during abiotic degradation of organic matter or hydrocarbon maturation.

Oxygen isotope ratios can, in certain circumstances, provide information on the temperature of mineral precipitation. During isotopic equilibrium precipitation, the $\delta^{18}\text{O}$ composition of a carbonate is related to both the temperature of precipitation and the composition of the fluid from which it precipitated. Assuming the pore waters to have been Jurassic seawater ($\delta^{18}\text{O} = 1.2\%$)¹² the temperature at which ankerite precipitated is estimated to be $\sim 115^\circ\text{C}$. Because the isotopic composition of the pore waters from which ankerite precipitates is unknown this temperature is unlikely to be accurate. However, the isotopic composition is consistent with precipitation from isotopically light, and/or hot pore waters^{1,3,6}. Interpretation of the isotopic data confirms petrographical observations. Ankerite authigenesis was late in the diagenetic sequence and occurred at a 'temperature' approaching the present reservoir temperature of 100°C (ref. 10). Precipitation probably involved CO_2 generated in a hotter, more deeply buried hydrocarbon-source rock in the Viking Graben and was related to Tertiary hydrocarbon migration into the reservoir^{10,13-15}.

Electron microprobe analysis reveals various chemical compositional trends within individual crystals. These trends reflect local fluctuating pore-water compositions during precipitation. However, the data as a whole exhibit a gradual decrease in CaCO_3 content with increasing depth (Fig. 1). It is suggested that this gross compositional change reflects evolving pore-water composition. When ankerite precipitation began throughout the reservoir pore waters were more calcium-rich. As hydrocarbons filled the reservoir, the precipitation of ankerite continued but pore-water composition changed progressively. The last ankerite precipitation occurred below the present oil-water contact, in relatively calcium-depleted pore waters.

Dissolution of, and equilibration with, earlier formed calcite or siderite respectively could have supplied the Ca^{2+} or Fe^{2+} required for ankerite precipitation. The replacement of calcite certainly may be implied from the high-minus cement porosities of the concretionary ankerites. However, ankerite, siderite and calcite coexist in the water. If dissolution and equilibration did take place, calcite dissolution would have become unfavourable during later precipitation when equilibrium was reached. This would explain the chemical compositional trends seen in the ankerite. Mg^{2+} , however, must have been introduced from outside the system, there being no precursor Mg^{2+} -bearing minerals present. In the Gulf Coast^{3,9}, ankerite formation in sandstones is related to mudrock diagenesis in the surrounding shales. The replacement of smectite by illite releases Mg^{2+} and Fe^{2+} into solution⁹, at temperatures which mark the onset of hydrocarbon migration^{9,13,14}. In both the hydrocarbon source rocks of the Viking Graben, and elsewhere in the North Sea, the replacement of smectite by illite has occurred and cation release is likely to have overlapped hydrocarbon generation^{13,14}.

It is postulated that ankerite began to form in the Ninian Field during hydrocarbon emplacement, as the invading pore waters reached equilibrium with the formation. In view of the sealed nature of the reservoir during hydrocarbon accumulation, fluid flow must have been convective¹⁶. Poikilotopic ankerite began to form by nucleating on and replacing preexisting authigenic calcite. Ubiquitous pre-filling rhombs also formed at this time. Ankerite precipitation continued below the oil-water contact as hydrocarbons arrived but was arrested as the oil column grew progressively while the reservoir filled. As Fe^{2+} and Mg^{2+} continued to arrive in the hydrocarbon-bearing fluids, pore-water compositions changed, leading to formation of progressively Ca^{2+} poorer ankerite with increasing depth (Fig. 1). The earlier formed cements now occur in the oil-bearing sandstones and the discrete pore-filling ankerite rhombs both in the oil and below the oil-water contact in the aquifer, having formed as saturated pore waters were displaced downwards by the accumulating hydrocarbons. Late stage ferroan carbonates and ankerites are reported from reservoirs elsewhere³⁻⁶. In view of the widespread association of altering smectites and maturing

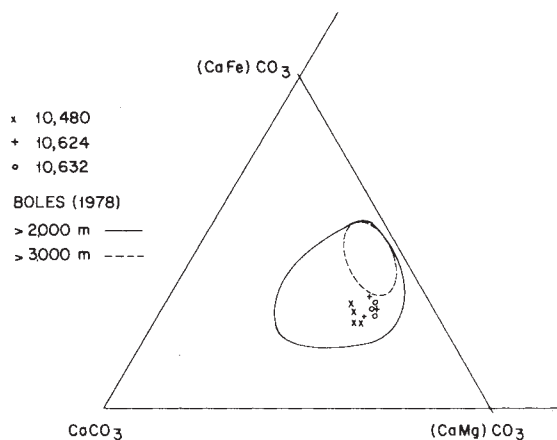


Fig. 1 Chemical compositional trends of ankerite from the Ninian Field. Data points are labelled in core or driller depths. The oil-water contact in 3/3-3 occurs at 10,503 feet driller depth (3201.3 m). Electron microprobe analyses were performed with a Links Systems Model 290 energy dispersive analyser fitted to a Cambridge Instruments Geoscan. The operating conditions were beam voltage 15 V, specimen current 2.5 nA and beam width $1\ \mu\text{m}$. The raw data were recalculated with a Links Systems ZAF 4 software package. Depths of the three cores in metres are 3,194.3 m, 3,238.2 m and 3,240.6 m, respectively.

source rocks^{9,13}, many may have a similar origin, although this has not been widely recognized¹⁷.

In the Gulf Coast, ankerite authigenesis is reported to be succeeded by the replacement of kaolinite by chlorite⁹. This reaction is also related to the release of Fe^{2+} and Mg^{2+} during smectite's transformation to illite⁹. In the Ninian Field and in Brent Group sandstones elsewhere, chlorite is only reported to occur in the Broom Formation¹¹ and it is not interpreted as replacing kaolinite¹¹. This could be due to the sediments not having reached the appropriate pore-water conditions. Alternatively, the Gulf Coast model may not be universally applicable as shown by the doubt concerning the original concept of the reaction's structural mechanism¹⁸.

In the Ninian Field, porosities at the oil-water contact are not reduced significantly by ankerite precipitation¹⁰. Indeed, ankerite is disseminated throughout the reservoir sequence. Authigenesis here has been promoted by metal cations and bicarbonate released from hydrocarbon-source rocks, and progressively arrested by the arrival of hydrocarbons. By contrast, with rapid hydrocarbon accumulation and a plentiful supply of bicarbonate and metal cations, ankerite-forming pore waters could be displaced into the aquifer thus concentrating its formation at the oil-water contact. Also, light hydrocarbon generation and emplacement before clay mineral reaction releasing cations

Table 1 Stable isotope ratios for authigenic ankerite

Well	Core depth (feet)	$\delta^{13}\text{C}$	$\delta^{18}\text{O}$	Precipitation temperature ($^\circ\text{C}$)
3/3-1	9,916	-9.7	-15.2	144
3/3-3	10,330	-7.1	-10.3	96
3/3-3	10,456	-13.6	-12.9	120
3/3-3	10,502	-9.4	-10.5	98

The samples were cleaned with 1% sodium hypochlorite¹⁹ to remove hydrocarbons and organic matter and reacted with phosphoric acid at 25°C for 2 h. The CO_2 evolved was analysed with a Micromass 903 mass spectrometer and the raw data corrected using the normal methods^{20,21}. Stable isotope ratios are reported as $\delta\%$ variations from PDB standard, corrected with a fractionation factor of 1.01109 (ref. 22). Hypothetical precipitation temperatures for ankerite were determined assuming ankerite-water fractionation to be similar to dolomite-water²³. Depth given in feet; equivalents in metres are 3,022.4 m, 3,148.6 m, 3,187.0 m and 3,201.0 m, respectively.

would restrict ankerite antithesis to below the oil-water contact. Thus, whilst high porosity and effective permeability might exist within both the oil column and the aquifer, connection between the two could become impaired. This possibility should be evaluated during reservoir development planning because any such transmissibility barrier could restrict the flow into the reservoir of either a natural aquifer or injection water. Both the loss of pressure support and the disruption of an injection pattern could adversely affect hydrocarbon recovery.

This research was carried out at the University of Hull whilst in receipt of NERC grant GT4/79/GS/39, and at the British Geological Survey (NERC) Stable Isotopes Unit. I thank the director of the BGS, Chevron Petroleum Ltd and Shell International Petroleum Maatschappij for permission to publish this paper. M. L. Coleman, R. S. Haszeldine and A. P. Heward commented on early versions of the manuscript.

Received 18 December 1984; accepted 27 March 1985.

1. Irwin, H., Curtis, C. D. & Coleman, M. L. *Nature* **269**, 209–213 (1977).
2. Matsumoto, R. & Iijima, A. *Sedimentology* **28**, 239–259 (1981).
3. Boles, J. R. *Contr. Miner. Petrol.* **68**, 13–22 (1978).
4. Uziemblo, N. & Petersen, H. *Bull. Am. Ass. petrol. Geol.* **67**, 562–563 (1982).
5. Woody, M. D. *Shale Shaker* **83**, 107–122 (1983).
6. Burley, S. D. *Clay Miner.* **19**, 403–440 (1984).
7. Dushcherer, W. *Oil Gas J.* 28 May, 143–150 (1984).
8. Franks, S. G. & Forester, R. W. *Bull. Am. Ass. petrol. Geol.* **68**, 478 (1984).
9. Boles, J. R. & Franks, S. G. *J. sedim. Petrol.* **49**, 55–70 (1979).
10. Albright, W. A., Turner, W. L. & Williamson, K. R. *Mem. Am. Ass. petrol. Geol.* **30**, 173–193 (1980).
11. Kantorowicz, J. D. *Clay Miner.* **19**, 359–375 (1984).
12. Shackleton, N. J. & Kennett, J. P. *Init. Rep. DSDP Leg 29*, 743–755 (1975).
13. Pearson, M. J., Watkins, D. & Small, J. S. *Dev. Sedim.* **35**, 665–675 (1982).
14. Pearson, M. J., Watkins, D., Pittion, J. L., Caston, D. & Small, J. S. *Geol. Soc. Lond. Spec. Publ.* **12**, 161–173 (1983).
15. Goff, J. C. *J. geol. Soc. Lond.* **140**, 445–474 (1983).
16. Haszeldine, R. S., Sampson, I. M. & Cornford, C. *Clay Miner.* **19**, 391–402 (1984).
17. Nash, A. J. & Pittman, E. D. *J. sedim. Petrol.* **45**, 258–265 (1975).
18. Nadeau, P. H., Tait, J. M., McHardy, W. J. & Wilson, M. J. *Clay Miner.* **19**, 67–76 (1984).
19. Forester, R. M., Sandberg, P. A. & Anderson, T. F. in *Living and Fossil Bryozoa* (ed. Larwood, G. A.) 79–94 (Academic, London, 1973).
20. Craig, H. *Geochim. cosmochim. Acta* **12**, 133–149 (1957).
21. Deines, P. *Int. J. Mass. Spectrom. Ion Phys.* **4**, 223–295 (1970).
22. Sharma, T. & Clayton, R. N. *Geochim. cosmochim. Acta* **29**, 1347–1354 (1965).
23. Fritz, P. & Smith, D. G. W. *Geochim. cosmochim. Acta* **34**, 1161–1173 (1970).

Anomalous $\delta^{13}\text{C}$ enrichment in modern marine organic carbon

Michael A. Arthur*, Walter E. Dean† & George E. Claypool†

*Graduate School of Oceanography, University of Rhode Island, Narragansett, Rhode Island 02882, USA

†US Geological Survey,*PO Box 25046, Denver, Colorado, USA

Marine organic carbon is heavier isotopically (^{13}C enriched) than most land-plant or terrestrial organic C¹. Accordingly, $\delta^{13}\text{C}$ values of organic C in modern marine sediments are routinely interpreted in terms of the relative proportions of marine and terrestrial sources of the preserved organic matter^{2,3}. When independent geochemical techniques are used to evaluate the source of organic matter in Cretaceous or older rocks, those rocks containing mostly marine organic C are found typically to have lighter (more-negative) $\delta^{13}\text{C}$ values than rocks containing mostly terrestrial organic C. Here we conclude that marine photosynthesis in mid-Cretaceous and earlier oceans generally resulted in a greater fractionation of C isotopes and produced organic C having lighter $\delta^{13}\text{C}$ values. Modern marine photosynthesis may be occurring under unusual geological conditions (higher oceanic primary production rates, lower P_{CO_2}) that limit dissolved CO_2 availability and minimize carbon isotope fractionation⁴.

In a recent compilation of geochemical analyses of samples of middle Cretaceous organic C-rich strata from numerous Deep Sea Drilling Project (DSDP) sites and several land sections⁵, we failed to observe a consistent relationship between organic carbon $\delta^{13}\text{C}$ and independent geochemical indicators of marine

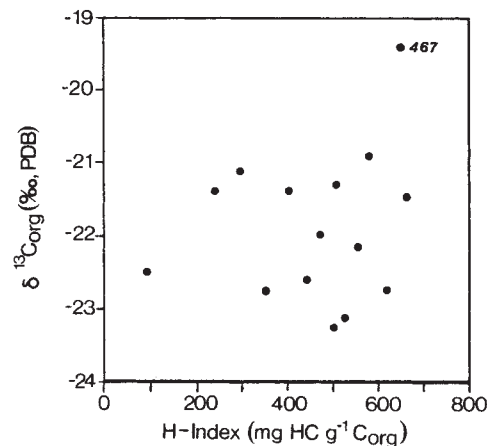


Fig. 1 Plot of $\delta^{13}\text{C}$ of organic C against Rock-Eval pyrolysis hydrogen index for organic C-rich sediments of Pleistocene-Miocene age from offshore California, DSDP Leg 63, Site 467. Hydrogen index is pyrolytic hydrocarbon yield per unit organic carbon in mg per g.

and terrestrial organic matter for samples older than Miocene. In fact, the Cretaceous sequences examined in the greatest detail usually showed the reverse of the expected relationship; that is, samples with the lightest (or apparently terrestrial) $\delta^{13}\text{C}$ values had chemical compositions indicating the greatest contributions of marine organic matter.

This apparently anomalous ^{13}C depletion in organic C of ancient sediments has been noted by others⁶, and is particularly troublesome in rocks older than Silurian⁷, or before the development of land plants. We conclude that organic C derived from marine plants in rocks that are Cretaceous or older typically have $\delta^{13}\text{C}$ values that are ~5–7‰ more negative than marine organic carbon in Holocene sediments.

Other techniques used to evaluate marine against terrestrial organic matter include atomic H/C ratios⁸, microscopic examination of isolated organic-matter particles⁹, abundance of phenolic lignin degradation products³, organic $^{15}\text{N}/^{14}\text{N}$ ratios¹⁰ and ^{13}C -nuclear magnetic resonance spectroscopy¹¹. In general, the relative proportions of marine and terrestrial organic matter indicated by these techniques are consistent with the proportions inferred from bulk organic C $\delta^{13}\text{C}$ values in modern marine sediments.

For the data sets that we have compiled⁵, the most common measure of H content is the H index from Rock-Eval pyrolysis, which has been shown to correlate with atomic H/C ratios¹². We plot organic C $\delta^{13}\text{C}$ against H index or atomic H/C of organic matter and observe either a positive correlation or no correlation for organic C rich sequences of Holocene-Miocene age. Figure 1 shows a typical lack of correlation for Holocene-Miocene sediments from offshore southern California⁵. Here, essentially all of the organic matter is from marine productivity induced by upwelling. This marine organic matter has $\delta^{13}\text{C}$ of ~–23 to –21‰, regardless of H content.

For Cretaceous and older organic C-rich sequences, the data commonly show a negative correlation between H index and $\delta^{13}\text{C}$ of organic matter. Figure 2 illustrates data from four different middle Cretaceous organic C-rich limestone sequences. These data sets were selected to minimize possible effects of differences in preparation procedures and lithology. In general, middle Cretaceous organic C-rich strata in the North American Basin of the western North Atlantic Ocean contain predominantly terrestrial organic matter, the abundance of which is greatest at those sites closest to the North American continental margin^{9,13}. In contrast, middle Cretaceous strata at DSDP sites adjacent to the African continental margin contain predominantly marine organic matter^{9,13}. Figure 2a shows $\delta^{13}\text{C}$ and H index data for interbedded Neocomian white limestones and black marlstones of the Blake-Bahama Formation¹⁴ cored at DSDP Site 603, one of the most terrestrially influenced

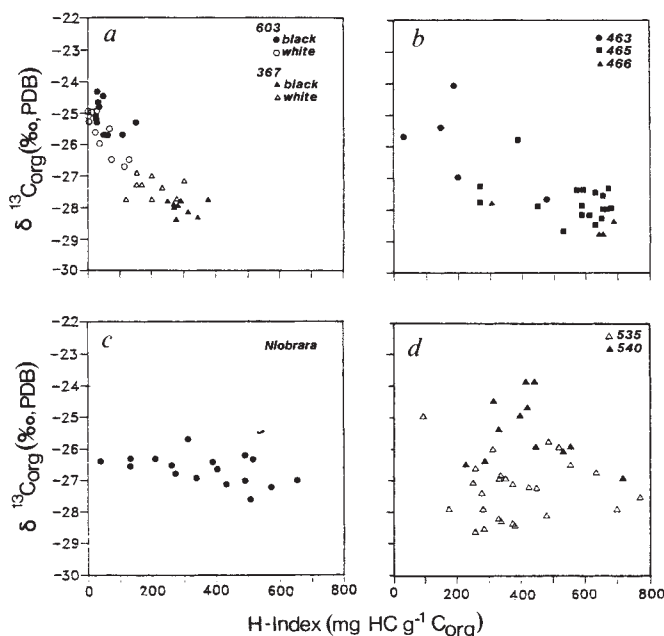


Fig. 2 Plots of $\delta^{13}\text{C}$ of organic C against Rock-Eval pyrolysis hydrogen index for *a*, samples of Neocomian black marlstone and white limestone from DSDP Sites 603, North American Basin, western North Atlantic and 367, Cape Verde Basin, southeastern North Atlantic⁵; *b*, samples of middle Cretaceous organic C-rich limestone from DSDP Sites 463, Mid-Pacific Mountains, and 465 and 466, southern Hess Rise, western North Pacific¹⁹; *c*, Upper Cretaceous Niobrara Limestone, Colorado⁵; and *d*, samples of middle Cretaceous limestone from DSDP Sites 535 and 540, eastern Gulf of Mexico²¹.

localities in the North American Basin. Also shown on Fig. 2a are data on coeval strata from DSDP Site 367 in the Cape Verde Basin off north-west Africa, which contains the most abundant marine organic matter of any DSDP Site in the North Atlantic^{9,13}. At both sites, much of the organic matter was redeposited by turbidity currents from continental slope settings^{15,16}. The $\delta^{13}\text{C}$ and H index data show distinctly different trends for these two sites (Fig. 2a). At Site 367, the organic matter in the black marlstone is H-rich and isotopically light ($\delta^{13}\text{C}$ of -28%) and, at Site 603, the organic matter in the black marlstones is H-deficient and isotopically heavier ($\delta^{13}\text{C}$ of -25%). The white limestones at both sites probably contain an oxidized residual mixture of marine and terrestrial organic matter, with possibly more marine influence at Site 367.

Figure 2b shows the relationship between $\delta^{13}\text{C}$ and H index for organic C-rich limestones of early Aptian to late Albian age from the Mid-Pacific Mountains and southern Hess Rise. These limestones were deposited on the flanks of seamounts and volcanic plateaus that were formerly under shallower water and under the equatorial high productivity zone during the middle Cretaceous¹⁷. The organic matter from these sites, especially from southern Hess Rise (Sites 465 and 466), is predominantly H-rich marine organic matter¹⁸. The relationship between isotope composition and H content of the organic matter is the same as that shown in Fig. 2a, that is, the most H-rich, most marine organic matter is isotopically the lightest. Figure 2c and d are data sets of samples from mid-Cretaceous limestones that apparently contain mostly marine organic matter. Figure 2c shows results of analyses of 17 samples from a quarry of the Coniacian-Santonian Niobrara Limestone deposited in the western interior seaway of the United States. The organic matter in these samples is mainly marine and the variation in H index is probably attributable to differing degrees of oxidation¹⁹. The tendency for the more H-rich organic matter to be isotopically lighter in the Niobrara may be a result of diagenesis rather than source of the organic matter. Samples of the early Cenomanian to late Albian limestone from DSDP Site 540 in the Gulf of

Mexico (Fig. 2d) have $\delta^{13}\text{C}$ values that range between ~ -27 to -24% with a H index range of 220–700. Other organic geochemical analyses²⁰ suggest that the organic matter is marine in origin, with lower H contents attributable to deposition under more oxidizing conditions. Samples of Cenomanian–Berriasian limestone from the nearby Site 535 also contain predominantly marine organic matter²¹ and have organic C $\delta^{13}\text{C}$ values that range from -28.7 to -25% . The differences between the $\delta^{13}\text{C}$ values from Sites 540 and 535 may be attributable to age differences and secular variations in $\delta^{13}\text{C}$ of the ocean–atmosphere C reservoirs, because the more-negative organic C $\delta^{13}\text{C}$ values at Site 535 are matched by correspondingly more-negative carbonate-C $\delta^{13}\text{C}$ values²⁰.

We conclude from numerous data sets, such as those illustrated in Fig. 2, that Cretaceous organic matter, interpreted to be largely marine by H richness or by other geochemical or optical indicators, has $\delta^{13}\text{C}$ values of -29 to -27% , and organic matter, interpreted to be largely terrestrial, has $\delta^{13}\text{C}$ values of -25 to -24% . Deposits of mixtures of terrestrial and marine organic matter, therefore, show a negative correlation between organic C $\delta^{13}\text{C}$ and H content (H index or atomic H/C). From this we conclude that Cretaceous marine organic matter was, on average, 5–7% lighter than Holocene marine organic matter.

There are several potential factors that could cause shifts in the $\delta^{13}\text{C}$ of marine organic matter, such as: (1) early to late diagenetic effects; (2) sea-surface temperature changes and their possible influence on CO_2 solubility and C isotope fractionation during photosynthesis; (3) changes in dominance of marine phytoplankton species and related isotope fractionation factors; (4) global or local changes in the $\delta^{13}\text{C}$ of oceanic dissolved CO_2 ; and (5) changes in general levels of productivity and the influence of CO_2 availability on isotope fractionation during planktonic photosynthesis. Thus, either the primary isotope composition of Cretaceous organic matter was the same or similar to that of the Neogene through Holocene and was modified during accumulation and diagenesis, or the isotope composition was different ($\sim 5\text{--}7\%$ more negative) for some primary reason.

Factors (1)–(4) above probably can account for only minor changes of 1 or 2% in the $\delta^{13}\text{C}$ of marine organic carbon⁵. Various diagenetic processes can alter the $\delta^{13}\text{C}$ of organic C by removing or adding ^{13}C -enriched or depleted fractions of the organic matter, but consistent unambiguous diagenetic trends in modern and Neogene sediments are difficult to document^{1,22}. Hatcher *et al.*¹¹ showed a 4% decrease in $\delta^{13}\text{C}$ of organic C of algal sapropelic sediment in a saline lake in Bermuda, but a diagenetic shift of this magnitude has not been observed in normal marine sediments. Cretaceous palaeotemperatures at low latitudes were as warm or warmer than those of today^{23,24}. If temperature change was the primary reason for the different $\delta^{13}\text{C}$ of organic carbon in the Cretaceous, then according to previous hypotheses^{25,26} we would expect marine organic C to have been isotopically heavier, not lighter as observed. In addition, evidence now suggests that there is not a direct simple relationship between surface water temperature and $\delta^{13}\text{C}$ of marine organic matter²⁷. Changes in C isotope fractionation as a result of plankton evolution or changes in the dominance of a particular phytoplankton group cannot be eliminated completely, but such changes are not supported by the Cenozoic palaeontological record and are considered unlikely to be the cause of the shift in the $\delta^{13}\text{C}$ of marine sedimentary organic matter. Secular variations in the $\delta^{13}\text{C}$ of marine carbonates²⁸ indicate that the $\delta^{13}\text{C}$ of total dissolved CO_2 in surface ocean water changed by 2–3% during the Cretaceous. However, the average Cretaceous carbonate $\delta^{13}\text{C}$ is $\sim 1.5\%$ heavier than modern pelagic carbonates, so that the effect of secular $\delta^{13}\text{C}$ variations would be to make Cretaceous marine organic matter heavier rather than lighter as observed. Kuspert²⁹ observed 4% lighter $\delta^{13}\text{C}$ in both organic and carbonate C of organic matter-rich beds in a Jurassic shale sequence, which he attributed to local contributions of oxidized organic C to the water column during episodes of anoxic stagnation. The main effect of local

or global $\delta^{13}\text{C}$ variations in dissolved CO_2 probably has been to induce variation of as much as 2–3‰ in the record of the isotope composition of marine organic matter through time.

The most probable cause of significantly lighter $\delta^{13}\text{C}$ of marine organic C in Cretaceous, compared with modern, sediments is the different set of conditions under which marine photosynthesis operated. Namely, the higher atmospheric CO_2 concentrations^{30,31} and the overall lower levels of oceanic productivity³² that prevailed during the Cretaceous. Photosynthetic C isotope fractionation is maximized under conditions that promote high CO_2 availability. In modern oceans, Antarctic diatoms with an abundant supply of CO_2 provided by upwelling of cold CO_2 -rich bottom waters in the Antarctic Divergence have more negative $\delta^{13}\text{C}$ than the plankton in low-latitude warm water with less available CO_2 (ref. 27). During much of the Cretaceous, the combination of low surface productivity and higher atmospheric CO_2 should have resulted in an ocean that had more available CO_2 and an increased fractionation of carbon isotopes in marine plankton.

Our interpretation of generally increased marine photosynthetic carbon isotope fractionation during Cretaceous (and earlier) time has several implications. The utility of $\delta^{13}\text{C}$ values alone for organic matter provenance studies should be considered carefully. The technique works reasonably well for Neogene–Quaternary sequences, but certainly not in the same way for Cretaceous sequences and probably not for much of the Phanerozoic. Variable marine photosynthetic fractionation of C isotopes in the past affects quantitative models of the C cycle. Most models assume a constant fractionation between CO_2 and organic matter and interpret $\delta^{13}\text{C}$ variations with time in terms of variations in the proportions of reduced and oxidized carbon reservoirs. Variable isotope fractionation during marine photosynthesis adds a significant complication to these models.

More work is necessary to document the preliminary conclusions that we offer here, both for the Cretaceous and for other times in the Phanerozoic. We emphasize that such geochemical results must be carefully collected and understood in the context of their facies and palaeogeographic settings.

Received 26 December 1984; accepted 27 February 1985.

- Deines, P. in *Handbook of Environmental Isotope Geochemistry* Vol. 1 (eds Fritz, P. & Fontes, J.) 329–406 (Elsevier, Amsterdam, 1980).
- Sackett, W. M. *Mar. Geol.* **2**, 173–185 (1964).
- Hedges, J. I. & Parker, P. L. *Geochim. cosmochim. Acta* **40**, 1019–1029 (1976).
- Deuser, W. G., Degens, E. T. & Guillard, R. R. L. *Geochim. cosmochim. Acta* **32**, 657–660 (1968).
- Dean, W. E., Arthur, M. A. & Claypool, G. E. *Mar. Geol.* **23** (in the press).
- Maynard, B. E. *Geology* **9**, 262–265 (1981).
- Towe, K. M. *Nature* **295**, 171 (1982).
- Stuerner, D. H., Peters, K. E. & Kaplan, I. R. *Geochim. cosmochim. Acta* **42**, 989–998 (1978).
- Summerhayes, C. P. *Bull. Am. Ass. Petrol. Geol.* **65**, 2364–2380 (1981).
- Peters, K. E., Sweeney, R. E. & Kaplan, I. R. *Limnol. Oceanogr.* **23**, 598–604 (1978).
- Hatcher, P. G., Spiker, R. E., Szeverenyi, N. B. & Maciel, G. E. *Nature* **305**, 498–501 (1983).
- Espitalie, J. ed. *Revue Inst. Fr. Pétrole* **23**, 23–42 (1977).
- Tissot, B., Demaison, G., Masson, P., Delteil, J. R. & Combaz, A. *Bull. Am. Ass. Petrol. Geol.* **64**, 2051–2063 (1980).
- Jansa, L. F., Enos, P., Tucholke, B. E., Gradstein, F. M. & Sheridan, R. E. in *Deep Drilling Results in the Atlantic Ocean: Continental Margins and Paleoenvironments* (eds Talwani, M., Hay, W. & Ryan, W. B. F.) 1–56 (Maurice Ewing Ser. 3, 1979).
- Dean, W. E. & Gardiner, J. V. in *Nature and Origin of Cretaceous Carbon-rich Facies* (eds Schlanger, S. O. & Cita, M. B.) 55–78 (Academic, London, 1982).
- DSDP Shipboard Scientific Party *Geotimes*, 16–18 (April 1984).
- Thiede, J., Dean, W. E. & Claypool, G. E. in *Nature and Origin of Cretaceous Carbon-rich Facies* (eds Schlanger, S. O. & Cita, M. B.) 79–100 (Academic, London, 1982).
- Dean, W. E., Claypool, G. E. & Thiede, J. *Org. Geochem.* **7**, 39–51 (1984).
- Pratt, L. M. *Bull. Am. Ass. Petrol. Geol.* **68**, 1146–1159 (1984).
- Patton, J. W., Choquette, P. W., Guannel, G. K., Kaltenback, A. J. & Moore, A. *Init. Rep. DSDP* **77**, 417–444 (1984).
- Herbin, J. P., Deroo, G. & Roucache, J. *Init. Rep. DSDP* **77**, 459–476 (1984).
- Galimov, E. in *Kerogen, Insoluble Organic Matter from Sedimentary Rocks* (ed. Durand, B.) 271–299 (Technip, Paris, 1980).
- Savin, S. A. *Rev. Earth Planet. Sci.* **5**, 319–355 (1977).
- Barron, E. J. *Earth Sci. Rev.* **19**, 305–338 (1983).
- Sackett, W. M., Eckelmann, W. R., Bender, M. L. & Be, A. W. H. *Science* **148**, 235–247 (1965).
- Fontugne, M. R. & Duplessy, J. C. *Earth planet. Sci. Lett.* **41**, 85–89 (1978).
- Rau, G. H., Sweeney, R. E. & Kaplan, I. R. *Deep-Sea Res.* **29**, 1035–1039 (1982).
- Scholle, P. A. & Arthur, M. A. *Bull. Am. Ass. Petrol. Geol.* **64**, 67–87 (1980).
- Kuspert, W. in *Cyclic and Event Stratification* (eds Einsele, A. & Seilacher, A.) 482–501 (Springer, Berlin, 1982).
- Barron, E. J. & Washington, W. M. *Palaeogeogr., Palaeoclimatol., Palaeoecol.* **40**, 103–133 (1982).
- Berner, R. A., Lasaga, A. C. & Garrels, R. M. *Am. J. Sci.* **283**, 641–683 (1983).
- Bralower, T. J. & Thierstein, H. R. *Geology* **12**, 614–618 (1984).
- Wong, W. W. & Sackett, W. M. *Geochim. cosmochim. Acta* **42**, 1809–1815 (1978).

Seasonal variations in natural abundance of ^{15}N in particles sinking to the deep Sargasso Sea

Mark A. Altabet & Werner G. Deuser

Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543, USA

Temporal variations in ^{15}N natural abundance have been used to study both nitrogen cycling and particle dynamics in the ocean¹. Changes in the $\delta^{15}\text{N}$ ($1,000 \times [({}^{15}\text{N}/{}^{14}\text{N})_{\text{sample}} / ({}^{15}\text{N}/{}^{14}\text{N})_{\text{atmospheric N}_2} - 1]$) of particulate matter are related, in part, to variations in the input of nitrogen to the euphotic zone that have been observed to regulate the downward transport of particles². Here we report the first long-term data set for seasonal variations in the $\delta^{15}\text{N}$ of sinking particulate matter sampled using a sediment trap. These variations co-vary with the magnitude of the particle flux. We propose that the $\delta^{15}\text{N}$ of sinking particles is a monitor for NO_3^- flux into the euphotic zone during the formation of these particles on a timescale directly related to the magnitude of the particle flux.

Deuser and Ross³ have reported seasonal variations in the flux of particulate matter into the deep ocean at a site in the Sargasso Sea. The fluxes of both biogenic and abiogenic components have been correlated with the total mass flux^{4–6}. It was suggested that this seasonal variation in particle flux occurred in response to a seasonal cycle in primary production, as has been observed by Menzel and Ryther⁷. The main forcing of the cycle in primary production was the convective mixing in winter of the upper water column and subsequent injection of nutrients into the euphotic zone. However, an internal record in the sediment trap samples of the processes directly related to primary production has not been found.

Considering the role of nitrogen in determining the magnitude of primary production, it seems that variations in the natural abundance of ^{15}N may be related to variations in particle flux. Seasonal changes in $\delta^{15}\text{N}$ values for suspended particulate organic nitrogen (PON) in a Gulf Stream warm-core ring have been reported¹. Low values for the $\delta^{15}\text{N}$ of euphotic-zone PON occurred when NO_3^- had been mixed into the euphotic zone. A similar observation was made by Wada and Hattori in the North Pacific Ocean⁹.

Measurements of ^{15}N natural abundance were made on particulate matter collected in a sediment trap (described previously⁶) moored at a depth of 3,200 m, ≈ 45 km south-east of Bermuda in the Sargasso Sea over a 6-yr period. The sediment trap was retrieved and re-deployed approximately once every two months so that the integrated flux over this period was sampled. No preservative was used, however, temperature at this depth is approximately 2.6°C . $\delta^{15}\text{N}$ measurements were made only on the $<37\text{-}\mu\text{m}$ size fraction, which contains $>90\%$ of the organic matter captured. Analyses for $\delta^{15}\text{N}$ were made using a Vg Micromass 602E dual-inlet double-collector mass spectrometer. Details of the sample preparation will be reported

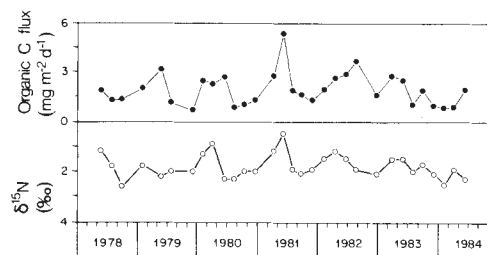


Fig. 1 The 6-yr record for organic carbon flux and the $\delta^{15}\text{N}$ of sinking particles at 3,200-m depth in the Sargasso Sea. Each time point represents an integration over the previous two months. $\delta^{15}\text{N}$ values have been plotted on an inverse scale.

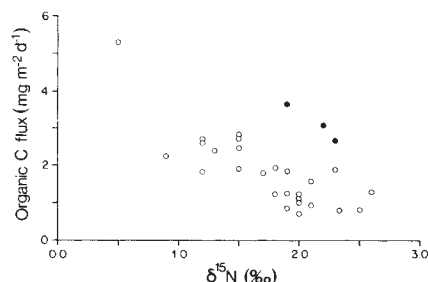


Fig. 2 Organic carbon flux versus $\delta^{15}\text{N}$. A linear regression of all points is statistically significant ($r = -0.62$, $n = 31$). Filled circles represent samples collected when the minimum in $\delta^{15}\text{N}$ preceded the maximum in organic carbon flux (see text). Excluding these points, $r = -0.80$ ($n = 28$).

elsewhere¹⁰. Values for $\delta^{15}\text{N}$ are relative to the ^{15}N content of atmospheric N_2 , with a reproducibility of $\pm 0.2\%$.

The time series data for both $\delta^{15}\text{N}$ and organic carbon flux are presented in Fig. 1. Note that $\delta^{15}\text{N}$ values are plotted on an inverse scale. Every year a maximum in organic carbon flux occurs in late spring to early summer, associated with a minimum in $\delta^{15}\text{N}$. In the autumn of 1983, there seems to be a small secondary maximum in organic carbon flux, corresponding to a minimum in $\delta^{15}\text{N}$. In 1981 and 1983, the minima in $\delta^{15}\text{N}$ co-occur exactly with the maxima in organic carbon flux. In 1979, 1980 and 1982, the minima in $\delta^{15}\text{N}$ precede the maxima in organic carbon flux by ~ 2 months. There is a significant correlation between organic carbon flux and $\delta^{15}\text{N}$ ($r = -0.62$, $n = 31$, Fig. 2). Part of the scatter can be attributed to the phase lag in 3 out of 5 years for which there is a complete record.

The annual cycle in particle flux may be considered to have two phases: a high-flux phase during which destratification has mixed nutrients into the euphotic zone and a low-flux phase characterized by undetectable nutrient concentrations in the euphotic zone. During the low-flux phase there is a small turbulent flux of nutrients across the seasonal thermocline, immediately used by phytoplankton. Because almost all the NO_3^- entering the euphotic zone is transformed into particulate nitrogen by this means, the potential for isotope fractionation is small. Assuming that the material collected in the sediment trap has undergone little degradation, the $\delta^{15}\text{N}$ of particulate nitrogen sinking during the low-flux phase should be similar to the $\delta^{15}\text{N}$ of NO_3^- below the euphotic zone. We do not yet have data for the $\delta^{15}\text{N}$ of NO_3^- in this region; nevertheless, the almost-constant $\delta^{15}\text{N}$ values for September–December of each year (except 1978) support the above hypothesis.

During the high-flux period, NO_3^- , which has been transported into the euphotic zone, is used by phytoplankton over several months, permitting isotope fractionation between NO_3^- and the particulate matter produced. As NO_3^- is depleted, the $\delta^{15}\text{N}$ of NO_3^- in the euphotic zone is expected to increase, possibly reaching high values before depletion occurs. The $\delta^{15}\text{N}$ of sinking particulate nitrogen is expected to be low in the first half of the high-flux period and, subsequently, to increase as NO_3^- is depleted from the euphotic zone. Integrated sampling over two months may not have provided sufficient resolution for the observation of this pattern.

Other factors will modify the predicted variation in time of the $\delta^{15}\text{N}$ of sinking PON. Wind events occurring throughout the high-flux period pump NO_3^- , relatively low in ^{15}N , into the euphotic zone, thereby mitigating the effect of NO_3^- uptake by phytoplankton. Consequently, the $\delta^{15}\text{N}$ of sinking PON tends to remain low during the high-flux period, as was observed for 1981. Variations in the sequence of wind events may be reflected in the time variation of the $\delta^{15}\text{N}$ of PON during the high-flux period.

These results have implications for the study of marine geochemical cycles. The seasonal decrease in $\delta^{15}\text{N}$ associated with an increase in particle flux represents in many respects a

'natural tracer experiment'. Studying seasonal variations in the $\delta^{15}\text{N}$ of other reservoirs of particulate matter (living and detrital) should yield valuable information on the exchanges and transformations that take place among them. Of particular interest is the interaction between the small suspended particles at depth (>150 m) and the downward flux of large particles⁸. Similarly, the mechanisms that package suspended matter in the euphotic zone into larger particles can be studied by examining covariations in $\delta^{15}\text{N}$ between these compartments.

If the sediments preserve a $\delta^{15}\text{N}$ record, these results suggest a means by which primary production in the ocean of the past can be studied. Such work would have ramifications for present studies of the relationships between world climate and global biological and chemical cycles. Further work in other regions is required to determine the generality of the correlation between particle flux and ^{15}N natural abundance.

We thank E. H. Ross for preparing the sediment-trap samples and J. J. McCarthy for use of the mass spectrometer. This work was supported in part by the Education Office of W.H.O.I., NSF grants OCE 80-22990 and OCE 83-14013 (J.J. McCarthy) and NSF grants OCE 76-21280, OCE 78-19813, OCE 80-24130 and OCE 82-19588 (W.G.D.). This is contribution no. 5913 from the Woods Hole Oceanographic Institution and no. 1039 from the Bermuda Biological Station.

Received 28 January; accepted 5 March 1985.

1. Altabet, M. A. & McCarthy, J. J. *Deep-Sea Res.* (in the press).
2. Eppley, R. W. & Peterson, B. J. *Nature* **282**, 677–680 (1979).
3. Deuser, W. G. & Ross, E. H. *Nature* **283**, 364–365 (1980).
4. Deuser, W. G., Brewer, P. G., Jickells, T. D. & Commeau, R. F. *Nature* **305**, 216–218 (1983).
5. Ittekkot, V., Deuser, W. G. & Degens, E. T. *Deep-Sea Res.* **31**, 1057–1069 (1984).
6. Deuser, W. G., Ross, E. H. & Anderson, R. F. *Deep-Sea Res.* **28A**, 495–505 (1981).
7. Menzel, D. W. & Ryther, J. H. *Deep-Sea Res.* **7**, 282–288 (1961).
8. Bacon, M. P., Huh, C.-A., Fleer, A. P. & Deuser, W. G. *Deep-Sea Res.* (in the press).
9. Wada, E. & Hattori, A. *Geochim. cosmochim. Acta* **40**, 249–251 (1976).
10. Nevins, J., Altabet, M. A. & McCarthy, J. J. *Analyt. Chem.* (in the press).

Photoadaptation of high Arctic ice algae

Glenn F. Cota

Marine Ecology Laboratory, Bedford Institute of Oceanography,
Department of Fisheries and Oceans, Dartmouth, Nova Scotia,
Canada B2Y 4A2

In aquatic systems, the layer that is suitable for positive net photosynthesis (the euphotic zone) is usually considered to extend from the surface down to the depth of penetration of 1% of the surface irradiance, which corresponds to $\sim 15 \mu\text{E m}^{-2} \text{s}^{-1}$ at solar noon during late summer in open waters in the high Arctic¹. In polar regions, vernal blooms of epontic algae (unicellular algae associated with the lower interface of sea ice whose photosynthetic characteristics are not well known^{2–4}) develop under conditions where they are only rarely or briefly exposed to light levels exceeding 1–2% of that incident at the surface. I report here that epontic algae from the Canadian Arctic show unusually high photosynthetic efficiencies normalized to pigment content, which increase with a decrease in the light levels at which the populations are growing. Photosynthesis was measurable at light intensities well below ambient (0.01% of surface irradiance). In the most shade-adapted populations⁵ under deeper snow cover, photosynthesis was optimal at light intensities close to the maximum ambient level, and inhibited at higher intensities. Furthermore, even after almost 2 months of exposure to elevated light levels (≈ 3 –4% of surface irradiance), biomass and productivity normalized to biomass were still lowest in algae from an area almost free of snow cover. Taken together, these results indicate that epontic algae from the high Arctic can be considered as an obligate shade flora genetically constrained to very low photon fluxes.

The main study site was ~ 1 km offshore from Sight Point on the southwestern tip of Resolute Bay, Northwest Territories, Canada ($74^{\circ}40'\text{N}$, $94^{\circ}54'\text{W}$). At this site, annual sea ice was

Table 1 Photosynthetic parameters for natural assemblages of ice algae from four light regimes defined by snow cover

Snow depth (cm)	Maximum ambient light ($\mu\text{E m}^{-2} \text{s}^{-1}$)	P_s^B	α	β ($\times 10^{-3}$)	P_m^B	I_m^*	I_k^*	I_b^*	I_c^*
20	≈ 5	0.047 (± 0.003)	0.094 (0.018)	0.44 (0.17)	0.046	2.7	0.5	107	NM
11	≈ 11	0.090 (± 0.003)	0.046 (0.003)	0.61 (0.12)	0.084	8.5	1.8	148	NM
3	≈ 23	0.045 (± 0.003)	0.010 (0.001)	0.04 (0.09)	0.044	25.0	4.3	1,125	0.18
0	≈ 50	0.025 (± 0.001)	0.007 (0.001)	0.01 (0.03)	0.025	23.0	3.3	2,500	NM

The initial slope is α ; P_s^B is the maximal photosynthetic rate without photoinhibition; β describes the slope at high intensities; P_m^B is the assimilation value; I_m , I_k , I_b and I_c are irradiances (see text). Standard deviations are given in parentheses for three primary (fitted) parameters. Units for P_s^B and P_m^B = $\text{mg C (mg chlorophyll } a)^{-1} \text{ h}^{-1}$; units for α and β = $\text{mg C (mg chlorophyll } a)^{-1} \text{ h}^{-1} (\mu\text{E m}^{-2} \text{s}^{-1})^{-1}$. NM, not measurable. * Units = $\mu\text{E m}^{-2} \text{s}^{-1}$ (E=Einstein).

~ 1.8 m thick and natural snow cover ranged from ~ 2 cm to 30 cm. Simple manipulations of the surface snow cover allowed considerable control over the light conditions of the epontic algae. Algal material was collected from the bottom few centimetres of ice with a SIPRE corer or an ice chisel. Algal assemblages with different light histories were taken from areas at discrete depths in the snow within a snow drift stabilized by a snow fence or from a 'cleared' patch of ice which was kept relatively free of snow with a snow blower. (The snow fence anchors the snow drift and prevents drift migration (much like sand dunes) during storms of erosion and/or deposition.) A transverse section of the two-sided drift is triangular, with the central apex at 30 cm height sloping down over 5–7 m to a height of 2–4 cm. Such a low-profile snow fence allows for an unusually high degree of control over the light environment within the stabilized snow field. Ambient light levels for the ice algal populations were inversely proportional to the depth of the overlying snow cover (Table 1). Algal samples were collected from 27 to 31 May 1984 after at least 6–7 weeks of growth in their respective light environments.

In all cases the algal assemblages were dominated by pennate diatoms, with *Nitzschia*, *Navicula*, *Amphipora* and *Pleurosigma* the most abundant genera. Although the communities were similar in species composition at each snow depth, standing stocks were markedly different. The species composition and levels of biomass were similar to those found in previous studies of high Arctic sea-ice communities^{6–9}.

Dense algal suspensions from the lower 2–5 cm of ice were diluted with two parts of surface sea water (water/ice = 2:1), and determinations of photosynthesis/irradiance relationships were done in a 'Photosynthetron'¹⁰ with $\approx 1.4 \mu\text{Ci NaH}^{14}\text{CO}_3 \text{ ml}^{-1}$ (1-h incubations at -1°C). Irradiance measurements were

made with a Licor 2π sensor before each experiment. Fluorimetric estimates¹¹ of chlorophyll *a* were used to normalize the rates of photosynthesis. Details of curve-fitting, parameter estimation and statistical considerations of photosynthesis/irradiance relationships have been described previously^{1,12,13}.

Photosynthesis/irradiance curves (Fig. 1a–d) show that very low ambient light levels are sufficient to saturate carbon fixation in the ice algae studied. The fitted and derived parameters for the curves and environmental data describing the four cases are summarized in Table 1. The populations from different snow depths (representing different light levels) display increasing degrees of photoadaptation that are consistent with their previous light environments. The initial slope, α , (a measure of photosynthetic efficiency) and the so-called adaptation parameter¹⁴, $I_k (= P_m^B/\alpha$, where B is chlorophyll biomass and m denotes maximal photosynthetic rate), both decrease by almost an order of magnitude over the natural range of ambient light levels and snow cover (Table 1). The ice algae from areas with deep snow cover (> 10 cm) exhibited exceptionally high photosynthetic efficiencies (α) at extremely low levels of irradiance (≈ 0.1 – $1.0 \mu\text{E m}^{-2} \text{s}^{-1}$ of photosynthetically active radiation, PAR).

Photoinhibition was pronounced only in the assemblages from regions of moderate and heavy snow cover, as shown by their relatively low values of $I_b (= P_s^B/\beta$, where s denotes the light-saturated photosynthetic rate), an index of photoinhibition¹² (Table 1). Values of I_b for Arctic phytoplankton range from $\sim 900 \mu\text{E m}^{-2} \text{s}^{-1}$ for assemblages from depths receiving 1% of the surface irradiance to $> 5,000 \mu\text{E m}^{-2} \text{s}^{-1}$ for populations from 30–50% light levels¹⁵. Except for the assemblage from the 'cleared' region, values of optimal irradiance, I_m , for the algal assemblages were very similar to the actual maximum light levels

Table 2 Parameters for photosynthesis-irradiance curves of various types of polar microalgae

Organism	P_s^B	α	$\beta (\times 10^{-3})$	P_m^B	I_m	I_k	I_c	Source
Arctic ice algae	0.052 (± 0.027)	0.039 (0.041)	0.27 (0.30)	0.050 (0.025)	15 (11)	2.5 (1.7)	≤ 0.2	Present study (all light levels)
Antarctic ice algae	—	0.023 (0.009)	0.20 (0.11)	0.103 (0.048)	24 (8)	4.8 (1.7)	—	Ref. 19 (two light levels)
Antarctic ice algae	—	—	—	—	≈ 20	≈ 10	≈ 1.0	Ref. 16
Arctic phytoplankton	1.32 (± 0.19)	0.011 (0.002)	0.17 (0.21)	1.22 (0.18)	587 (117)	117 (15)	6.1	Ref. 1 (50% light level)
Arctic phytoplankton	1.29 (± 0.51)	0.008 (0.002)	1.18 (0.79)	0.79 (0.21)	359 (89)	112 (34)	1.4	Ref. 1 (1% light level)
Antarctic phytoplankton	—	0.009 (0.007)	—	1.93 (1.35)	117 (33)	31 (9)	—	Ref. 24
Antarctic benthic diatoms	—	0.022 (0.005)	Negligible	0.21 (0.03)	—	11 (1)	—	Ref. 23 (one light level)

Mean values ($\pm$ s.d.) are shown. Units have been standardized with the following conversion factors to correspond to those given in Table 1: $1 \text{ W m}^{-2} = 4.66 \mu\text{E m}^{-2} \text{s}^{-1}$; $1 \text{ klux} = 17.5 \mu\text{E m}^{-2} \text{s}^{-1}$; $1 \text{ ft candle} = 0.19 \mu\text{E m}^{-2} \text{s}^{-1}$. I_m is optimal irradiance; I_k , the 'light-adaptation' index; I_c , the compensation intensity in units of $\mu\text{E m}^{-2} \text{s}^{-1}$ (see Table 1 and text).

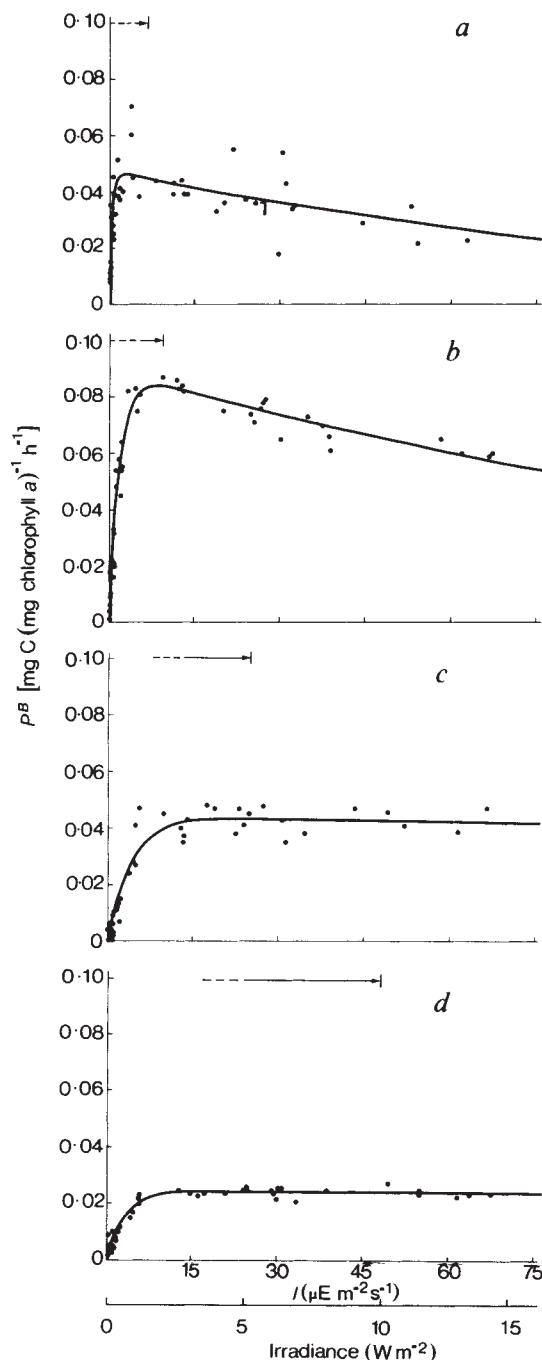


Fig. 1 Photosynthesis/irradiance curves for four populations of ice algae from different snow depths (or light levels): *a–d*, 20, 11, 3 and 0 cm, respectively. Maximum *in situ* light levels measured at solar noon for each nominal snow depth are indicated by the arrows in the upper portion of each panel.

recorded *in situ* at those snow depths (Table 1). Close correspondence of the derived irradiance parameters, I_m and I_k , to their respective light environments indicates advanced states of photoadaptation in these populations.

Maximum photosynthetic rates, P_m^B , measured at all snow depths were low, but they are representative of the lower end of the range of values reported by others studying ice algae^{4,6,7,16–19}. Previous work²⁰ and limited evidence from the present study suggests that assimilation values may vary directly with nutrient status. The values of P_m^B for the algal populations also parallel the 1984 distributional pattern of biomass

(chlorophyll) with snow cover, when the lowest levels were found under little or no snow. Maximum apparent growth rates calculated from increases in chlorophyll concentration over a few days and assuming no losses, were ~0.22 and 0.14 doubling per day for the combined data from moderate to heavy snow cover and little or no snow, respectively. That the algae with little or no snow cover were unable to achieve relatively more rapid growth and/or production rates suggests that they represent an obligate shade flora, genotypically adapted to very low photon flux densities²¹.

These data indicate extreme degrees of photoadaptation and, in fact, the derived irradiance parameters (I_m , I_k and I_c) describing photosynthetic performance at low light levels are the lowest values of this sort reported for marine microalgae to date^{4,21,22}. (The term adaptation is used here 'in the physiological sense, that is, as a physiological adjustment to the surrounding conditions'²³.) Table 2 shows some of the most relevant information on the photosynthetic responses of other natural assemblages of polar marine microalgae^{1,16,19,23,24}. Phytoplankton from both poles apparently are adapted to light levels that barely overlap with the maximal levels of irradiance in epontic systems: for example, even the relatively low I_k for Antarctic phytoplankton²⁴ is slightly above the highest values of ambient light that we measured under slight (2–4 cm) snow cover (Fig. 1c). With respect to photosynthetic efficiency of light utilization, the epontic algae under heavy snow cover (Table 1) yield values of α that are 4–10 times higher than those of polar phytoplankton (Table 2). In addition, the compensation intensity, I_c ($= |R^B/\alpha|$, where R^B is the ordinate intercept), for the ice algae was considerably lower than values for arctic phytoplankton (Table 2).

Previous work on epontic algae has emphasized the importance of snow cover in regulating the light environment^{2–4,6,7,25}. Removal of snow cover over established populations of ice algae may result in photoinhibition, pigment degradation or habitat destruction (that is, melting of the soft interfacial ice) from excessive absorptive heating^{2–4,16,25}. The present experimental results emphasize the importance of photoinhibition, and preliminary calculations on the magnitude of absorptive heating by this intense chlorophyll maximum lend support to the idea of habitat alteration.

The ice algae studied here have adapted to a point where they can flourish in a marginal environment at near freezing temperature with very low levels of light. In terms of overall efficiency of light conversion, their optimal irradiances are well within the dynamic range proposed by Radmer and Kok²⁶ for the most energetically efficient photosynthesis. Moreover, high Arctic ice algae are apparently not able to function effectively in light conditions exceeding ambient levels, yet in the Antarctic similar ice algae are able to achieve very high levels of biomass approaching theoretical limits (that is, maximum chlorophyll per unit area) in some cases¹⁸.

This work was supported by the National Sciences and Engineering Research Council of Canada, the Office for Energy Research and Development and the Polar Continental Shelf Project (Department of Energy, Mines and Resources). I thank J. Anning, T. Siferd and D. Rudderham for technical assistance and T. Platt, M. R. Lewis, W. G. Harrison and N. Watson for critical comment.

Received 29 November 1984; accepted 19 February 1985.

1. Platt, T., Harrison, W. G., Irwin, B., Horne, E. P. & Gallegos, C. L. *Deep Sea Res.* **29**, 1159–1170 (1982).
2. Apollonio, S. *Arctic* **14**, 197–200 (1961).
3. Apollonio, S. *Arctic* **18**, 118–122 (1965).
4. Bunt, J. S. *Nature* **199**, 1255–1257 (1963).
5. Steemann Nielsen, E. & Jørgensen, E. G. *Physiologia Pl.* **21**, 401–413 (1968).
6. Alexander, V., Horner, R. & Clasby, R. C. *Inst. mar. Sci. Rep.* R74–4 (University of Alaska, 1974).
7. Horner, R. & Schrader, G. C. *Arctic* **35**, 485–503 (1982).
8. Cross, W. *Arctic* **35**, 13–27 (1982).
9. Hsiao, S. I. C. *Arctic* **33**, 768–793 (1980).
10. Lewis, M. R. & Smith, J. C. *Mar. Ecol. Prog. Ser.* **13**, 99–102 (1983).
11. Holm-Hansen, O., Lorenzen, C. J., Holmes, R. W. & Strickland, J. D. H. *J. Cons. Int. Explor. Mer* **30**, 3–15 (1965).

12. Platt, T., Gallegos, C. L. & Harrison, W. G. *J. mar. Res.* **38**, 687-701 (1980).
13. Gallegos, C. L. & Platt, T. *Can. Bull. Fish. Aquat. Sci.* **210**, 103-112 (1981).
14. Talling, J. F. *New Phytol.* **56**, 133-149 (1957).
15. Gallegos, C. L., Platt, T., Harrison, W. G. & Irwin, B. *Limnol. Oceanogr.* **28**, 698-708 (1983).
16. Bunt, J. S. *Antarct. Res. Ser.* **1**, 27-31 (1964).
17. Bunt, J. S. & Lee, C. C. *J. mar. Res.* **28**, 304-320 (1970).
18. Palmisano, A. C. & Sullivan, C. W. *Polar Biol.* **2**, 171-177 (1983).
19. Palmisano, A. C., Soohoo, J. B. & Sullivan, C. W. *J. Phycol.* (in the press).
20. Vedernikov, V. I. *Oceanology* **15**, 482-485 (1975).
21. Richardson, K., Beardall, J. & Raven, J. A. *New Phytol.* **93**, 157-191 (1983).
22. Fogg, G. E. *Phil. Trans. R. Soc. Lond.* **279**, 27-38 (1977).
23. Palmisano, A. C., White, D. C., Smith, G. A., Stanton, G. R. & Burckle, L. H. *J. Phycol.* (in the press).
24. Jacques, G. *Polar Biol.* **2**, 27-33 (1983).
25. Sullivan, C. W. *et al. Antarct. J. U.S.* **18**, 177-179 (1983).
26. Radmer, R. J. & Kok, B. in *Encyclopedia of Plant Physiology, New Ser. Vol. 5* (eds Trebust, A. & Avron, M.) 125-135 (Springer, Berlin, 1977).

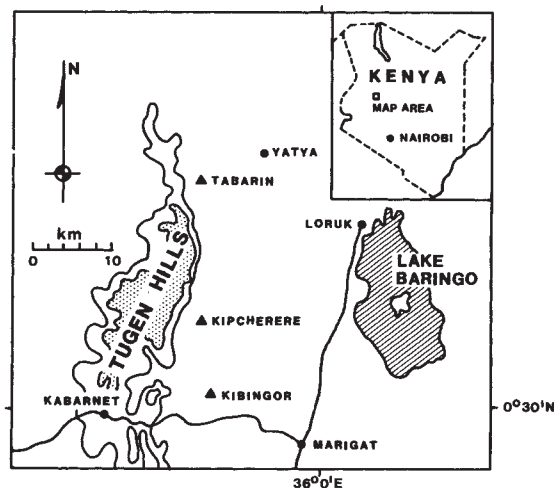


Fig. 1 Map of the area to the west of Lake Baringo, showing the Tugen Hills and the location of Tabarin.

Early hominid from Baringo, Kenya

Andrew Hill

Department of Anthropology, Harvard University,
Peabody Museum, Cambridge, Massachusetts 02138, USA

Palaeontological evidence and comparative molecular studies of modern hominoids suggest that major evolutionary changes occurred in the African Hominoidea between 14 Myr and 4 Myr¹⁻³. Unfortunately, very few relevant fossils are known from that time interval⁴⁻¹³. Rocks exposed in the fault scarps of the Tugen Hills in the north Kenya Rift Valley west of Lake Baringo are important as the major known outcrops of fossiliferous sediments spanning this otherwise poorly known time period^{13,14}. As reported here, a new fossil hominid from Tabarin, within this sequence, establishes the presence of the family in Africa as much as 5 Myr ago. Before this find the oldest well-documented occurrence of hominids was the *Australopithecus afarensis* material from Laetoli, Tanzania¹⁵. The Tabarin specimen is up to one million years nearer the divergence point between hominids and the African apes. In several features it resembles *A. afarensis* rather than other hominoids.

Tabarin (Baringo Palaeontological Research Project site, BPRP K077; UTM 36N ZR 186844) is a newly discovered fossil locality about 2 km north of Rondonin, north-west of Lake Baringo (Fig. 1). It is an outcrop of the Chemeron Formation¹⁶ *sensu* Martyn (refs 17, 18; see also refs 13, 19). At the base of the local section is a flow of the Kaparaina basalt which separates the sediments from those of the Lukeino Formation beneath. The basalt is overlain by some 20 m of fine- to medium-grained sediments, followed by a tuffaceous unit ~10 m thick. Much of this is ignimbritic and bakes the underlying sediments, but the upper part is a coarse pumice tuff, and pumiceous patches also occur at the base. After successive units comprising 20 m of mainly yellow and orange silts and sands, there is a dark brown coarsely bedded ferruginous conglomerate containing poorly sorted igneous clasts; this is fossiliferous, and on the basis of location and matrix it seems that much of the surface fossil material also derives from this unit. The succession of overlying sediments, some also containing fossils, continues towards the Saimo Fault of the main Tugen Hills range.

Isotopic determinations (K/Ar) on the tuff unit underlying the main fossiliferous horizon give dates of 4.96 ± 0.03 Myr (on anorthoclase) and 5.25 ± 0.04 Myr (on sanidine) (G. Curtis and R. Drake, personal communication). Because the fossiliferous sediments are apparently conformable on the surface of the dated tuff, it is reasonable to suppose that the hominid is not appreciably younger than these isotopic dates. The faunal evidence is entirely compatible with this date and independently places an upper limit on the age of 4.15 Myr.

The fossil fauna from Tabarin is abundant and varied (Table 1), including lacustrine animals and those that would be found along a lake margin and in its catchment area. Particularly crucial for dating purposes is the suid *Nyanzachoerus jaegeri*, and the proboscideans, notably *Anancus kenyensis*. The most

specific fossil is *N. jaegeri*, which is found in sediments older than 4.15 Myr (corrected for new constants) in the Mursi Formation at the Omo^{20,21}, and at Aterir which is dated at about the same age^{14,20}. It is not found in the Hadar Formation^{20,22}. It is, however, known from the Aramis Member and the Beeryada beds that underlie the Kalaloo beds of the Sagantole Formation in the Middle Awash Complex²³⁻²⁵. These units lie beneath the Cindery Tuff dated at 3.8-4.0 Myr^{11,26}. It does not occur in the Laetoli fauna at 3.7 Myr^{15,20}. *Anancus kenyensis* is not known later than 4 Myr (despite ref. 27) and is not part of the Laetoli fauna at 3.7 Myr, although it is found in the older Lower Laetoli Beds (J. Harris, personal communication). In Ethiopia it is not found in the Hadar Formation²². It is known in the Omo sequence only in the Mursi Formation, dated at older than 4.15 Myr^{21,28}. Kalb *et al.* report a single uncollected specimen from the Kalaloo beds of the Sagantole Formation, Middle Awash Complex²⁵, which is stratigraphically immediately above the Cindery Tuff horizon. The rest of the Tabarin fauna is compatible with this interpretation (see Table 1).

The hominid specimen (KNM-TH 13150; Fig. 2) was found by Kiptalam Cheboi. It was a surface find but its situation and matrix unequivocally link it to the conglomeratic unit and the rest of the fauna.

From other sites in Africa, several of them in the Tugen Hills succession, are a very few other late Miocene and Pliocene hominoid specimens, which cannot reliably be allocated to taxa⁴⁻¹³. Most definite Pliocene hominid material comes from Laetoli in Tanzania dated at 3.7 Myr¹⁵, and from the Afar region of Ethiopia ranging in age from ~3.3 Myr to 2.8 Myr^{29,30}. The material shows a considerable variation in size and morphology, and has been described as a single species, *Australopithecus afarensis*, with this variation being explained as sexual dimorphism³¹. In addition, the species is postulated to be the ancestral form from which all later hominids were derived^{32,33}. Despite, or perhaps because of, its relative abundance, the material has been subject to various other interpretations: for example, some acknowledge that it represents the common ancestor of later hominids yet regard it as nothing more than a subspecies of *Australopithecus africanus*³⁴. Others differ primarily in believing that the initial hominid radiation had already taken place. Of these, some imply that the *A. afarensis* material represents only one species but suggest that it was directly ancestral to, or shared a common ancestor with, the robust australopithecines³⁵; others are convinced that the variation in the sample indicates that there are two or more sympatric species, the ancestors of both robust australopithecines and later *Homo*³⁶⁻³⁹.

The Tabarin specimen closely resembles relevant specimens from the smaller end of the size range of *A. afarensis*, for example

Table 1 Fossil fauna from Tabarin and Sagatia

	Tabarin	Sagatia
Mollusca		
Bivalvia	×	
Pisces		
Siluriformes		
cf. <i>Clarias</i>	×	×
Reptilia		
Chelonia		
Testudinidae		×
Trionychidae	×	×
Pelomedusidae	×	×
Crocodylia	×	×
Mammalia		
Primates		
Cercopithecidae		
Papionini		×
Hominidae		
cf. <i>Australopithecus afarensis</i>	×	
Carnivora		
Hyaenidae		×
Felidae		
cf. <i>Panthera</i>		×
Small species		×
Mustelidae		
<i>Enhydriodon</i> cf. <i>campani</i>		×
Another species; family indeterminate		×
Proboscidea		
Gomphotheriidae		
<i>Anancus kenyensis</i>	×	×
Elephantidae		
cf. <i>Primelephas gomphotheroides</i>	×	×
Deinotheriidae		
<i>Deinotherium bozasi</i>	×	×
Perissodactyla		
Equidae		
<i>Hipparion</i>	×	×
Rhinocerotidae	×	×
Artiodactyla		
Suidae		
<i>Nyanzachoerus jaegeri</i>	×	×
Hippopotamidae	×	×
Bovidae		
Several species	×	×
Rodentia		
Hystriidae		
<i>Hystrix</i>	×	

Fossil fauna from Tabarin and additional species found at Sagatia, a site 1.5 km to the south at the same stratigraphical level as Tabarin. There are also well-preserved leaf impressions and coprolites. Tabarin has an archaic elephant, probably the same species as that found at other Chemeron Formation sites, but which is not known from Hadar or Laetoli, and which Maglio⁴⁴ considers to be typical of ~5 Myr. The papionine is consistent with an early Pliocene age (M. G. Leakey, personal communication). The *Enhydriodon* resembles a form found at Kanapoi. The hyaenid resembles one from Kanapoi which also occurs at Kaiso (J. Barry, personal communication).

A.L. 288-li and A.L. 128. It is smaller than the type specimen (LH-4) and the larger material from Hadar; it is also smaller than the Lothagam mandible (KNM LT-329). In detail it has several characters which typify *A. afarensis* rather than other hominoids: on the mandibular corpus these include a large buccal eminence and hollowing of the lateral contour. The teeth have a strong buccal flare. The differential proportions of mesial and buccal roots produce a serrate pattern, and there is a bifid mesial root apex on M₃ (refs 33, 40–43).

However, many of the parts diagnostic of *A. afarensis* are not available on this specimen, and while time and geography should not be used taxonomically, it is true that increasing distance in time or space increases the probability that a specimen, particularly one preserving a small number of morphological traits, represents something significantly different from other known forms. As the Tabarin specimen is up to a million years older than the oldest specimens attributed to *A. afarensis*, increasing the record of the Hominidae as a whole back in time by 25%,

©1985 Nature Publishing Group

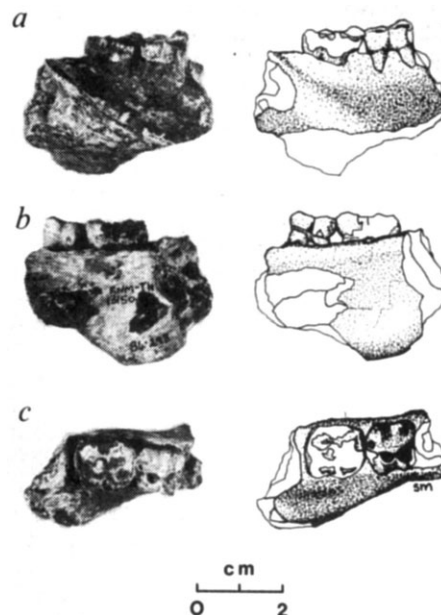


Fig. 2 The hominid specimen from Tabarin (KNM-TH 13150): buccal (a), lingual (b) and occlusal (c) views. The specimen consists of a fragment of right mandibular corpus with M₁ and M₂, and some of the alveolus and a root fragment of P₄. The mesial break extends from the P₄ alveolus diagonally and distally to below M₂. Part of the buccal surface of the corpus is lost ventrally. This damaged area links the mesial to the distal fracture, which extends distal to M₂. Weathering has removed a uniform and extremely thin layer of surface bone from much of the lingual surface, although the contours are preserved. The whole of the cortex has been lost over a small area of the lingual surface extending from the mesial fracture to below mid-M₂. The fractured margins, including the broken edge of the P₄ root, are worn. There is no obvious postmortem distortion. The basal surface of the corpus is present distal to M₂, where the corpus is 26.9 mm in height perpendicular to the alveolar margin. The root of the ramus is present, with the oblique line extending at ~45° to the alveolar margin into a flared buccal prominence. The prominence and extra-molar sulcus lose distinction below M₁, where the buccal face becomes slightly concave mesially. From the alveolus P₄ can be seen to have a bifurcated root, the mesial being more buccally placed than the distal. M₁ is intact but for the loss of the mesial margin of the protoconid, and measures 11.1 mm mesiodistally and 10.4 mm buccolingually. Wear has exposed small dentine islands on metaconid and hypoconulid. Larger exposures on protoconid and hypoconid are almost united. The lingual border is steep, with a shallow lingual groove. There is a buccal groove on the more inclined and flared buccal surface. Most of the occlusal enamel of M₂ has been lost through postmortem damage, as has that on the buccal surface and on the lingual face of the entoconid. A small section of the contact facet for M₃ remains distally. The tooth measures 13.1 mm mesiodistally, and 11.4 mm buccolingually. The mesiodistal dimension is slightly reduced by interproximal wear with M₁, where the contact facet measures 4.5 mm buccolingually. As in M₁, the lingual face is steep with a lingual groove; the buccal face slopes and has a buccal groove. Mesial roots on both M₁ and M₂ are broader than distal roots. Although weathering has removed the surface of the M₃ alveolus, the form of the remaining bone suggests that the mesial root was directed somewhat posteriorly and had a bifid apex.

its attribution may change when further material is available. More detailed discussions of the relationship of the Tabarin hominid to other late Miocene and Pliocene hominoids will appear elsewhere.

The specimen is important in that it resembles later known hominids from a time estimated by comparative molecular studies to be very close to the divergence point with African apes^{1–3}. Additional material from Tabarin and other new sites in the area will be critical to understanding the details of the early phase of hominid evolution and discriminating between

the various hypotheses that have arisen around specimens described as *A. afarensis*. Further material may show the same large range of variation as these later specimens or it may provide more explicit evidence of an early radiation in the Hominidae between 5 Myr and 3.7 Myr.

This work forms part of the Baringo Palaeontological Research Project, based at Harvard University, and carried out jointly with the National Museums of Kenya. It was financed by a NSF grant (BNS 81-40818) to David Pilbeam, John Barry and Kay Behrensmeyer, and by a grant from the Louise Brown Foundation to David Pilbeam and A.H. I thank Kiptalam Cheboi, John Kimengich and Sally McBrearty for their help, and Richard Leakey and the National Museums of Kenya for much logistical support. I thank the Government of the Republic of Kenya for research permission; Mary Leakey for allowing me to examine the Laetoli hominid material; Garniss Curtis and Robert Drake for allowing me to quote the results of isotopic age determinations which they performed; and I particularly thank Steven Ward for his advice, and John Barry, Greg Chapman, Jay Kelley and David Pilbeam for their helpful comments on the manuscript. Sally McBrearty drew the figures.

Received 3 August 1984; accepted 12 February 1985.

1. Sarich, V. M. & Wilson, A. C. *Science* **158**, 1200-1203 (1967).
2. Andrews, P. & Cronin, J. E. *Nature* **297**, 541-546 (1982).
3. Sibley, C. G. & Ahlquist, J. E. *J. molec. Evol.* **20**, 2-15 (1984).
4. Patterson, P. *Nature* **212**, 577-581 (1966).
5. Patterson, P. & Howells, W. W. *Science* **156**, 64-66 (1967).
6. Patterson, P., Behrensmeyer, A. K. & Sill, W. D. *Nature* **266**, 918-921 (1970).
7. Bishop, W. W. & Chapman, G. R. *Nature* **226**, 914-918 (1970).
8. Pickford, M. *Nature* **256**, 279-284 (1975).
9. Pickford, M., Johanson, D. C., Lovejoy, C. O., White, T. D. & Aronson, J. L. *Am. J. phys. Anthropol.* **60**, 337-346 (1983).

10. Pickford, M. in *New Interpretations of Ape and Human Ancestry*, 421-439 (Plenum, New York, 1983).
11. Clark, D. et al. *Nature* **307**, 423-428 (1984).
12. Ishida, H., Pickford, M., Nakaya, H. & Nakano, Y. *African Study Monographs Suppl. Issue* **2**, 73-85 (1984).
13. Hill, A. et al. *J. hum. Evol.* (submitted).
14. Bishop, W. W., Chapman, G. R., Hill, A. & Miller, J. A. *Nature* **233**, 389-394 (1971).
15. Leakey, M. D. et al. *Nature* **262**, 460-466 (1976).
16. McCall, G. J. H., Baker, B. H. & Walsh, J. in *Background to Evolution in Africa*, 191-220 (University of Chicago Press, 1967).
17. Martyn, J. E. *Nature* **215**, 476-477 (1967).
18. Martyn, J. E. thesis, Univ. London (1969).
19. Hill, A., Curtis, G. & Drake, R. in *Sedimentation in the African Rift System* (Blackwells, Oxford, in the press).
20. Harris, J. M. & White, T. D. *Trans. Am. phil. Soc.* **69**, 1-128 (1979).
21. Brown, F. H. & Lajoie, K. R. *Nature* **229**, 483-485 (1971).
22. Johanson, D. C., Taieb, M. & Coppens, Y. *Am. J. phys. Anthropol.* **57**, 373-402 (1982).
23. Kalb, J. E. et al. *Nature* **298**, 25-29 (1982).
24. Kalb, J. E., Oswald, E. B., Mebrate, A., Tebedge, S. & Jolly, C. J. *Newsl. Stratigr.* **11**, 95-127 (1982).
25. Kalb, J. E. et al. *J. Vertebr. Palaeont.* **2**(2), 237-258 (1982).
26. Hall, C. M., Walter, R. C., Westgate, J. A. & York, D. *Nature* **308**, 26-31 (1984).
27. Coppens, Y., Maglio, V. J., Madden, C. T. & Beden, M. in *Evolution of African Mammals*, 336-367 (Harvard University Press, 1978).
28. Beden, M. in *Earliest Man and Environments in the Lake Rudolf Basin*, 193-208 (University of Chicago Press, 1976).
29. Brown, F. H. *Nature* **300**, 631-633 (1982).
30. Schmitt, T. J. & Nairn, A. E. M. *Nature* **309**, 704-706 (1984).
31. Johanson, D. C., White, T. D. & Coppens, Y. *Kirtlandia* **28**, 1-14 (1978).
32. Johanson, D. C. & White, T. D. *Science* **202**, 321-330 (1979).
33. White, T. D., Johanson, D. C. & Kimbel, W. H. *S. Afr. J. Sci.* **77**, 445-470 (1981).
34. Tobias, P. V. *Palaeont. afr.* **23**, 1-17 (1980).
35. Falk, D. & Conroy, G. C. *Nature* **306**, 779-781 (1983).
36. Senut, B. *L'Humérus et ses Articulations chez les Homínides Plio-pléistocène* (CNRS, Paris, 1982).
37. Olson, T. R. in *Aspects of Human Evolution*, 99-128 (Taylor & Francis, London, 1981).
38. Coppens, Y. in *Recent Advances in the Evolution of Primates*, 1-9 (Pontifical Academy of Sciences, The Vatican, 1983).
39. Tardieu, C. *L'Articulation du Genou* (CNRS, Paris, 1983).
40. Johanson, D. C. et al. *Am. J. phys. Anthropol.* **57**, 403-451 (1982).
41. White, T. D. & Johanson, D. C. *Am. J. phys. Anthropol.* **57**, 501-544 (1982).
42. Johanson, D. C., White, T. D. & Coppens, Y. *Am. J. phys. Anthropol.* **57**, 545-603 (1982).
43. Ward, S. C., Johanson, D. C. & Coppens, Y. *Am. J. phys. Anthropol.* **57**, 605-630 (1982).
44. Maglio, V. J. *Trans. Am. phil. Soc.* **63**, 1-149 (1973).

Chromosome Y-specific DNA in related human XX males

David C. Page*, Albert de la Chapelle† & Jean Weissenbach‡

* Whitehead Institute for Biomedical Research, Nine Cambridge Center, Cambridge, Massachusetts 02142 and Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

† Department of Medical Genetics, University of Helsinki, 00290 Helsinki 29, Finland

‡ Unité de Recombinaison et Expression Génétique (INSERM V. 163, CNRS LA 271), Institut Pasteur, 27 rue du Dr Roux, 75015 Paris, France

Human 'XX males' are sterile males whose chromosomes seem to be those of a normal female. About 1 in 20,000 males has a 46, XX karyotype, and most cases are sporadic, that is, they are without familial clustering¹. It has long been argued that maleness in XX males may result from the undetected presence of a small, testis-determining fragment of the Y chromosome², and there is strong evidence for this in sporadically occurring XX males³⁻⁵. Indeed, the genomes of three of four sporadic XX males tested were found to contain certain Y-specific DNA sequences⁶. A pedigree in which three XX males occur has been interpreted as being consistent with autosomal recessive inheritance of maleness^{7,8}, and it has been argued that the basis of XX maleness in this family is fundamentally different from that in the sporadic cases⁹. However, we report here that these related XX males, like the sporadic cases, contain portions of the Y chromosome. The portion of the Y chromosome present in one of the three XX males differs from that present in the other two.

In one of the few families reported to contain two or more XX males, the pattern of inheritance suggests a dominant male-determining locus segregating according to an autosomal mode¹⁰. Affected siblings¹¹ and affected identical twins¹² have also been described. The family reported here has been described

previously^{7,8}. Briefly, two XX males are second cousins, and they are distantly related to a third XX male via a common male ancestor (Fig. 1a). The mothers of two of these XX males are related in several ways. It has been argued that an autosomal⁸ or 'pseudoautosomal'⁹ recessive male-determining gene is segregating in this family.

In the present study, DNAs from three related XX males and their relatives were tested for the presence of certain Y-specific sequences using probes derived from the human Y chromosome. Some of these Y-specific DNA sequences were previously found to be present in, and others absent from, the genomes of sporadic XX males⁶. We have reported previously the absence in these three related XX males of the Y-specific 15-kilobase (kb) *TaqI* fragment at the X-Y homologous locus *DXYS1* (ref. 13). DNAs prepared from cultured fibroblasts or from peripheral blood leukocytes were digested with the restriction endonuclease *TaqI* and gel transfers¹⁴ of the resulting fragments were hybridized with radiolabelled DNA probes detecting Y-specific *TaqI* fragments. Table 1 summarizes the results of these tests and Fig. 1b, c shows autoradiograms of some of the original data. All three XX males carry certain Y-specific sequences. The two XX males who are second cousins, X-1 and X-2, seem to carry similar if not identical portions of the Y chromosome; that is, their genomes contain the two Y-specific *TaqI* fragments detected by probe 47c, but they are negative for all other Y-specific sequences tested. (In this respect, they resemble the sporadic XX male '3' previously reported by Guellaen et al.⁶.) X-3, the distantly related XX male, is positive not only for the two Y-specific *TaqI* fragments detected by 47c, but also for some of the Y-specific *TaqI* fragments detected by probes 50f2, 52d and 118. (The patterns observed were identical with those seen in sporadic XX male '4' reported by Guellaen et al.⁶.) None of the three XX males is positive for all of the Y-specific sequences. We conclude that each carries only a part of the Y chromosome.

By this test, there is no evidence of abnormality at the DNA sequence level in any of the relatives. The fathers of the XX males and a brother, X-4, are positive for all of the Y-specific sequences tested, while the mothers and a sister, X-5, are negative for all of the Y-specific sequences. Moreover, the intensities

of the Y-specific bands (relative to autosomal or X-specific bands) in the fathers are similar to those seen in normal unrelated males (data not shown). Thus, as the fathers all have a normal male karyotype, it is likely that the Y-specific DNA sequences they display are present in normal copy number and only on the Y chromosome. As in *Sxr* mice^{15,16}, 'sex reversal' in these humans may be the result of an X;Y translocation. However, unlike *Sxr*, duplication of the testis-determining portion of the Y chromosome in carrier males is apparently not involved.

We conclude that these three related XX males are males because, like the three sporadic XX males reported by Guellaen *et al.*⁶, they carry a testis-determining fragment of the Y chromosome. Note that the patterns of Y-specific fragments shown by these related XX males are among the patterns shown by sporadic XX males. In the case of each of the three XX males, a new mutation must have occurred in the father, during or before the meiotic divisions. That mutation apparently involved breaking the Y chromosome and perhaps translocating one of the resulting pieces to the X chromosome. We detected no Y-specific sequences in any of the three mothers. (Guellaen *et al.*⁶ previously reported the same finding in the mother of one sporadic XX male.) These results seem incompatible with a maternal contribution to XX maleness in this family; such a contribution had been suggested by weak positivity for the H-Y antigen in the mothers⁸. If there is an inherited defect predisposing to XX maleness in this family, it is operating in the fathers, in whom Y chromosomes are broken.

The two closely related XX males (X-1 and X-2) carry a similar if not identical portion of the Y chromosome, and they are related through males only. Thus, it is possible that the Y chromosome common to their fathers is susceptible to the sort of gross mutation described above, perhaps at a particular site on the Y chromosome. Indeed, a Y-linked mutation predisposes to reciprocal X;Y translocation in the mouse¹⁷. If a Y-linked mutation is operating in the fathers of the two closely related XX males, the occurrence of XX maleness in a distant relative (X-3), related through a lineage that includes females as well as males, may be merely coincidental. This is consistent with the finding that X-3 carries a different, albeit overlapping, fragment of the Y chromosome.

Using X-linked restriction fragment length polymorphisms, we have shown previously that most, if not all, XX males, including the three studied here, inherit one X chromosome from their father and one from their mother¹³. However, like many XX males, X-3 does not express his father's allele for the dominant, X-linked marker Xg. (Xg segregation is uninformative in the other two XX males.) As reported above, X-3 has inherited part of his father's Y chromosome. This is reminiscent

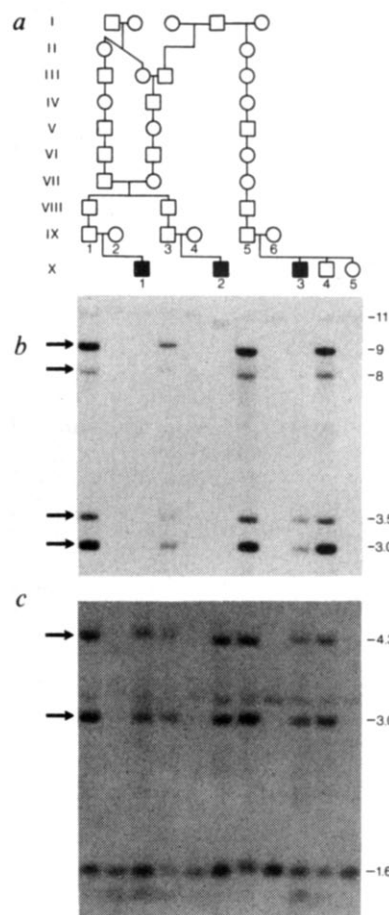


Fig. 1 *a*, Simplified paternal pedigree of XX males X-1, X-2 and X-3 (black squares) (from ref. 8). *b*, *c*, Hybridization of *TaqI*-digested DNAs from the XX males and their relatives with 50f2 (*b*) and 47c (*c*). Each lane corresponds to the individual immediately above that lane in the pedigree shown in *a*. DNA samples were digested with *TaqI*, electrophoresed on agarose gels, transferred¹⁴ to Pall Biotrans (1.2- μ m pores) nylon paper and hybridized with radiolabelled probes largely as described previously¹³. However, following transfer, the air-dried papers (DNA-coated side up) were exposed to germicidal (short-wave) ultraviolet light at an intensity of 400 μ W cm⁻² for 1 min; they were not baked. The arrows on the left indicate the Y-specific *TaqI* fragments detected. The sizes of restriction fragments (in kilobase pairs) are shown on the right.

Table 1 Y-specific DNA sequences in XX males and their relatives

Individual	Probes							
	47c	50f2	52d	118	27	49f	12f3	pDP34*
Normal male	2	4	3	8	1	3	1	1
Normal female	0	0	0	0	0	0	0	0
IX-1 (GM2672) father	2	4	3	ND	ND	ND	ND	1
IX-2 (GM2671) mother	0	0	0	ND	ND	ND	ND	0
X-1 (GM2626) XX male	2	0	0	0	0	0	0	0
IX-3 (GM2624) father	2	4	3	ND	ND	ND	ND	1
IX-4 (GM2625) mother	0	0	0	ND	ND	ND	ND	0
X-2 (GM2670) XX male	2	0	0	0	0	0	0	0
IX-5 (LGL118) father	2	4	3	ND	ND	ND	ND	1
IX-6 (LGL117) mother	0	0	0	ND	ND	ND	ND	0
X-3 (LGL115) XX male	2	2	2	4	0	0	0	0
X-4 (LGL116) brother	2	4	3	ND	ND	ND	ND	1
X-5 (LGL119) sister	0	0	0	ND	ND	ND	ND	0

Genomic DNAs from normal unrelated males and females and from the indicated members of the pedigree in Fig. 1a were digested with *TaqI*, blotted and hybridized with probes detecting Y-specific sequences. Methods were as described for Fig. 1b, c. The number of male-specific *TaqI* fragments detected by each probe are shown. ND, not done.

* The data for probe pDP34 (*DXYS1*) are from ref. 13.

of our finding of a sporadic XX male who expresses his father's allele for 12E7, a Y-linked marker, but fails to express his father's Xg allele⁵. Both cases can be explained by an interchange of a portion of the Y chromosome with a portion of the paternal X chromosome². However, such an interchange cannot easily account for XX males X-3's apparent failure to express his father's allele for the dominant, X-linked marker *Xm* (ref. 1), which maps to the long arm of the X chromosome¹⁸; Xg maps to the short arm. It is possible that the *Xm* typing of this family was in error. Unfortunately, as *Xm* antiserum is no longer available, the typing cannot be repeated.

Taken together, our findings and those of Guellaen *et al.*⁶ suggest that, though variable in size, the segment of the Y chromosome present is similar in at least some familial and sporadic XX males. It is likely that that segment of the Y chromosome carries the genetic information which is male-determining.

This study was aided by grants from the NIH, the Sigrid Jusélius Foundation, the Folkhalsan Institute of Genetics and the Academy of Finland. We thank Laura Brown for assistance, George Church for technical advice, and David Botstein, in whose laboratory some of these experiments were performed.

Received 28 December 1984; accepted 4 March 1985.

- de la Chapelle, A. *Hum. Genet.* **58**, 105-116 (1981).
- Ferguson-Smith, M. A. *Lancet* **ii**, 475-476 (1966).
- Evans, H. J., Buckton, K. E., Spowart, G. & Carothers, A. D. *Hum. Genet.* **49**, 11-31 (1979).
- Magenis, R. E. *et al. Hum. Genet.* **62**, 271-276 (1982).
- de la Chapelle, A., Tippet, P. A., Wetterstrand, G. & Page, D. *Nature* **307**, 170-171 (1984).
- Guellaen, G. *et al. Nature* **307**, 172-173 (1984).
- de la Chapelle, A., Schröder, J., Murros, J. & Tallqvist, G. *Clin. Genet.* **11**, 91-106 (1977).
- de la Chapelle, A., Koo, G. C. & Wachtel, S. S. *Cell* **15**, 837-842 (1978).
- Burgoyne, P. *Nature* **307**, 109 (1984).
- Kasdan, R. *et al. New Engl. J. Med.* **288**, 539-545 (1973).
- Minowada, S. *et al. Clin. Genet.* **15**, 399-405 (1979).
- Nicolis, G. L. *et al. Am. J. Med.* **52**, 482-491 (1972).
- Page, D. C. & de la Chapelle, A. *Am. J. hum. Genet.* **36**, 565-575 (1984).
- Southern, E. M. *J. molec. Biol.* **98**, 503-517 (1975).
- Singh, L. & Jones, K. W. *Cell* **28**, 205-216 (1982).
- Evans, E. P., Burtenshaw, M. D. & Cattanach, B. M. *Nature* **300**, 443-445 (1982).
- Eicher, E. M. in *Prospects for Sexing Mammalian Sperm* (eds Amann, R. P. & Seidel, G. E.) (Colorado Associated University Press, Boulder, 1982).
- Berg, K. & Bearn, A. G. *Rev. Genet.* **2**, 341-362 (1968).

Table 1 Results of crosses of STS-deficient C3H/An with normal mice

Female	Cross	No. of offspring			
		Females		Males	
		STS ⁺	STS ⁻	STS ⁺	STS ⁻
*C3H/An (STS ⁻)	BALB/c (STS ⁺)	13	0	17	0
*BALB/c (STS ⁺)	C3H/An (STS ⁻)	6	0	8	0
*C3H/An (STS ⁻)	Feral mouse (STS ⁺)	3	0	5	0
*C3H/An (STS ⁻)	C57B (STS ⁺)	Not tested		7	0
*(C3H/An × C57B) F ₁ (STS ⁺)	C3H/An (STS ⁻)	10	13	13	15
*(C3H/An × C57B) F ₁ (STS ⁺)	C57B (STS ⁺)	16	0	17	0
C3H/An (STS ⁻)	(C3H/An × BALB/c) F ₁ (STS ⁺)	2	6	2	4
C3H/An (STS ⁻)	(BALB/c × C3H/An) F ₁ (STS ⁺)	1	6	2	1

STS assays were carried out on sonicated spleen extracts using ³H-dehydroepiandrosterone sulphate as substrate, according to Shapiro *et al.*³². Pure-breeding STS⁺ mice have STS activity of >250 pmol per h per mg protein and pure-breeding STS⁻ mice (C3H/An) have <7 pmol per h per mg protein. The STS activity of STS⁺ hybrid mice varied from 70 to 160 pmol per h per mg protein.

* Unpublished data from Balazs *et al.*¹².

When C3H/An STS-deficient mice are crossed with STS normal animals in various combinations, the results seem compatible with simple autosomal inheritance of STS deficiency (Table 1). However, one litter from a cross of an STS-deficient female with a supposed pure-breeding STS normal male yielded one STS-deficient female. If the exceptional female was X0, it would suggest that STS deficiency follows an unusual form of sex-linked inheritance. Unfortunately, materials were not available for cytological analysis of the exceptional animal nor could the male parent be tested for possible heterozygosity.

We therefore instituted crosses of C3H/An STS-deficient males with STS normal X0 mice marked with the tabby (*Ta*) gene. The X-linked tabby marker permits separation of mice carrying *Ta*, in either the hemizygous or homozygous condition, from heterozygotes and wild-type mice by its characteristic coat markings. From these crosses, three classes of offspring are expected with regard to tabby and sex: *Ta*/+♀♀, +/0♀♀ and *Ta*/Y♂♂. Table 2 gives the results of these crosses with respect to tabby, sex and STS level. If the STS variant we are studying is autosomal, there should be no differences in STS levels between the three classes of offspring. On the other hand, if the STS variant lies on the X-chromosome, then the X0 progeny should be STS-deficient, in contrast to the normal or intermediate values for the *Ta*/+ and *Ta*/Y animals. In fact, the X0 animals show STS values not significantly different from their deficient male parent. The absence of STS-deficient animals among 15 *Ta*/Y and *Ta*/+ siblings argues against the possibility that the X0 parents are heterozygous for STS deficiency at an autosomal locus. These results demonstrate X-linkage of the C3H/An STS deficiency.

The studies reported here (Table 1) and elsewhere¹² using the C3H/An STS-deficient mouse strain in various crosses yield results compatible with simple autosomal inheritance of the deficient *STS* gene in the C3H/An strain. However, when deficient C3H/An males are crossed to STS normal X0 mice, the segregation pattern observed in the offspring demonstrates X-linkage of the *STS* deficient gene (Table 2). These apparently contradictory results are best explained by postulating a functional Y-linked *STS* gene that undergoes obligatory recombination with its X-linked allele. As both the C3H/An and BALB/c Y chromosomes exhibit recombination with the X-linked *STS*

X-linkage of steroid sulphatase in the mouse is evidence for a functional Y-linked allele

Elisabeth Keitges, Martha Rivest, Marcello Siniscalco* & Stanley M. Gartler

The Center for Inherited Diseases, Departments of Medicine and Genetics, SK-50, University of Washington, Seattle, Washington 98195, USA

* Memorial Sloan-Kettering Cancer Center and Department of Cell Biology and Genetics, Sloan-Kettering Division, Graduate School of Medical Sciences, Cornell University, New York, New York 10021, USA

In the human there is an X-linked gene affecting steroid sulphatase (STS) activity which, when deficient, is associated with X-linked congenital ichthyosis^{1,2}. The gene (*STS*) is located on the distal tip of the short arm³⁻⁶ and is only partially inactivated when it is on the inactive X-chromosome⁷⁻¹⁰. In the mouse, the genetics of *STS* are not clear; the results of one study using XX:X0 oocyte comparisons indicated X-linkage¹¹, but three other studies using STS variants have produced segregation data compatible with autosomal linkage of murine *STS*¹²⁻¹⁴. Here we present the results of STS assays of crosses of deficient C3H/An male mice¹² to normal X0 animals which demonstrate X-linkage of *STS* in the mouse and indirectly indicate the existence of a functional *STS* allele on the Y-chromosome which undergoes obligatory recombination during meiosis with the X-linked allele.

Table 2 Levels of STS activity resulting from crosses of STS⁺ X0 to STS⁻ XY mice

	STS activity (pmol per h per mg protein)				
	Parents		Offspring		
Sex chromosomes:	STS ⁺ X0	STS ⁻ XY	X0†	XX	XY
Tabby genotype:	Ta/0	+/Y	+/0	Ta/+	Ta/Y
	198	25	27	310	189
	119	42	23	260	188
	217	52	36	162	236
	281		62	274	176
	198		16	114	234
	162		10	244	140
	134				131
					313
					258
Mean STS value	187	40	29	227	207

Each STS value represents an individual mouse. Tabby X0 mice (F₁ mice from C57BL/6J × CBA/Ca crosses) were obtained from Jackson Laboratories (Bar Harbor, Maine). The STS assay is a modification of the technique of McNaught and France³³. Ear punches of 2 mm diameter were placed in 100 µl of buffer (0.1 M Tris-HCl pH 7.2) and frozen on dry ice. The samples were then thawed and sonicated on ice for 5 s at 250 W and placed on ice. This cycle was repeated four more times. 'Cold' oestrone sulphate (4 ng) and ³H-oestrone sulphate (2 µCi, specific activity 52.5 Ci mmol⁻¹; NEN) were added to 50 µl of the extract on ice. A zero-time sample of 26 µl of the assay mixture was added to 25 µl of 0.1 M NaOH. The rest of the reaction mixture was incubated at 37 °C for 40 min, then the reaction was stopped by adding 25 µl of 0.1 M NaOH. Petroleum ether (350 µl) was added to each sample and the mixtures vortexed for 30 s. The samples were placed on dry ice and an aliquot (100 µl) of the organic phase was removed and added directly to 5 ml of toluene/Omnifluor for radioisotope counting. Protein assays were done according to the Lowry method³⁴.

* The values reported here are for the three STS⁻ males used in the above crosses. All STS⁻ mice (C3H/An) have low STS levels which do not overlap with those of either the pure-breeding STS⁺ strain or the F₁ animals. Duplicate assays varied by <10%.

† One X0 offspring was examined cytologically and the modal chromosome count was found to be 39.

Y-linked STS gene. However, another closely linked gene, gene (Table 1, crosses shown in lines 7 and 8) the recombinational property postulated here is not a unique characteristic of the C3H/An strain.

Ultrastructural studies of crossing-over in the XY pair of various mammals, including the mouse¹⁵, suggest that there is partial synapsis of the X and Y, followed by chiasma formation. A model including a single obligatory crossover between the X and Y¹⁶⁻¹⁸ would predict that genes distal to the crossover will be transmitted to both male and female offspring and appear to be autosomally inherited. The *Sxr* ('sex-reversed') mouse¹⁹ is a precedent for this possibility since the *Sxr* rearrangement is passed equally to male and female offspring^{20,21}. *Sxr* has been mapped to the distal tip of the X and Y coincident with the proposed X-Y pairing region of the mouse. Accordingly, the STS locus should map to the same region. It should be pointed out that our data on the STS locus in the mouse directly contradict the theory of Ashley²², who suggests that synapsis between the X and Y chromosomes is non-homologous and that regular crossing-over does not occur between the X and Y chromosomes.

In man, the X-linked STS locus maps to the distal tip of the short arm of the X, which is the presumed pairing region of the human sex chromosomes³⁻⁶. The X-linked recessive pattern of inheritance of human STS deficiency precludes a functional

MIC2, which codes for the red blood cell antigen 12E7, does have an expressible allele on the Y chromosome in human²³. Obligatory recombination proximal to this locus appears to be precluded by results from studies of a polymorphism of 12E7 which shows a complex sex-limited expression of variation in 12E7 levels²⁴. Therefore, if there is an obligatory crossover between the human X and Y, it would have to be distal to 12E7 and STS. These observations do not exclude the possibility of occasional exchange between the X and Y in human, which has been shown to occur in the origin of some XX males²⁵⁻³⁰.

With functional alleles on the X and the Y chromosomes in the mouse, dosage compensation is not required to effect equality of expression of X-linked alleles in females and males³¹. In fact, the similarity of STS values in X0 STS⁺ parents and XX STS⁺ daughters seems to exclude the STS locus from X-inactivation. We know from coat colour markings that the tabby gene in the XX daughters is being randomly inactivated; if the STS locus followed the same pattern, then the STS values of the XX daughters would be approximately half those of the X0 mothers (Table 2). Definitive evidence for or against X-inactivation of the STS locus will require some form of cloning study in heterozygotes.

Thus, we have shown by crossing STS normal X0 mice with C3H/An STS-deficient males, that the STS deficiency of the C3H/An mice is controlled by an X-linked gene. This result, in conjunction with previous data suggesting autosomal inheritance of the C3H deficiency, implies the existence of an STS allele on the Y chromosome that undergoes obligatory recombination.

We thank Dr Eva Eicher for helpful comments. This work was supported by Genetic Center grant GM15253 and HD16782 from the National Institute of General Medical Sciences. E.K. was supported by postdoctoral training grant GM07454. S.M.G. is the recipient of a NIH Career Award.

Received 12 December 1984; accepted 28 February 1985.

- Jobsis, A. G. *et al. Ned. Tijdschr. Geneesk.* **120**, 1980 (1976).
- Shapiro, L. J., Weiss, R., Webster, D. & France, J. T. *Lancet* **i**, 70-72 (1978).
- Tiepolo, L. *et al. Hum. Genet.* **54**, 205-206 (1980).
- Muller, C. R., Westerveld, A., Migl, B., Franke, W. & Ropers, H. H. *Hum. Genet.* **54**, 201-204 (1980).
- Mohandas, T., Shapiro, L. J., Sparkes, R. S. & Sparkes, M. C. *Proc. natn. Acad. Sci. U.S.A.* **76**, 5779-5783 (1979).
- Mohandas, T., Sparkes, R. S., Hellkuhl, B., Grzeschik, K. H. & Shapiro, L. J. *Proc. natn. Acad. Sci. U.S.A.* **77**, 6759-6763 (1980).
- Muller, C. R., Migl, B., Traupe, H. & Ropers, H. H. *Hum. Genet.* **54**, 197-199 (1980).
- Chance, P. F. & Gartler, S. M. *Am. J. hum. Genet.* **35**, 234-240 (1983).
- Ropers, H. H. *et al. Hum. Genet.* **57**, 354-356 (1981).
- Migeon, B. R. *et al. Nature* **299**, 838-840 (1982).
- Gartler, S. M. & Rivest, M. *Genetics* **103**, 137-141 (1983).
- Balazs, I., Purrello, M., Rocchi, M. & Siniscalco, M. *Cytogenet. Cell Genet.* **32**, 251 (1982).
- Erickson, R. P., Harper, K. & Kramer, J. M. *Genetics* **105**, 181-189 (1983).
- Keinanen, B. M., Nelson, K., Daniel, W. L. & Roque, J. M. *Genetics* **105**, 191-206 (1983).
- Solari, A. J. *Int. Rev. Cytol.* **38**, 273-317 (1974).
- Ferguson-Smith, M. A. *Lancet* **ii**, 475-476 (1966).
- Burgoyne, P. S. *Hum. Genet.* **61**, 85-90 (1982).
- Polani, P. E. *Hum. Genet.* **60**, 207-211 (1982).
- Cattanach, B. M., Pollard, C. E. & Hawkes, S. G. *Cytogenetics* **10**, 318-337 (1971).
- Singh, L. & Jones, K. W. *Cell* **28**, 205-216 (1982).
- Evans, E. P., Burtenshaw, M. D. & Cattanach, B. *Nature* **300**, 443-445 (1983).
- Ashley, T. *Hum. Genet.* **67**, 372-377 (1984).
- Goodfellow, P. *et al. Nature* **302**, 346-349 (1983).
- Goodfellow, P. N. & Tippet, P. *Nature* **289**, 404-405 (1981).
- de la Chapelle, A. *Hum. Genet.* **58**, 105-116 (1981).
- Evans, H. J., Buckton, K. E., Spowart, G. & Carothers, A. D. *Hum. Genet.* **49**, 11-31 (1979).
- Pierella, P., Craig, I., Bobrow, M. & de la Chapelle, A. *Hum. Genet.* **59**, 87-88 (1981).
- Wieacker, P., Voiculescu, J., Muller, C. R. & Ropers, H. H. *Cytogenet. Cell Genet.* **35**, 72-74 (1983).
- de la Chapelle, A., Tippet, P. A., Wetterstrand, G. & Page, D. *Nature* **307**, 170-171 (1984).
- Guellaen, G. *et al. Nature* **307**, 172-173 (1984).
- Crocker, M. & Craig, I. *Nature* **303**, 721-722 (1983).
- Shapiro, L. J., Cousins, L., Fluharty, A. L., Stevens, R. L. & Kihara, H. *Pediat. Res.* **11**, 894-897 (1977).
- McNaught, R. W. & France, J. T. *J. Steroid Biochem.* **13**, 363-373 (1980).
- Lowry, O. H., Rosebrough, N. J., Lewis Farr, A. & Randall, R. J. *J. biol. Chem.* **193**, 265-275 (1951).

Does interocular transfer occur in visual navigation by ants?

Rüdiger Wehner & Martin Müller

Department of Zoology, University of Zurich,
Winterthurerstrasse 190, CH-8057 Zurich, Switzerland

If an animal that learns to respond to a visual stimulus presented to only one eye can continue to respond accurately when the stimulus is presented to the other eye, it is said to exhibit interocular transfer (IOT). IOT has been studied extensively in many groups of vertebrates¹⁻¹¹, but there is only one conclusive report of IOT in an invertebrate (*Octopus*)¹². Here we demonstrate that an insect, the desert ant *Cataglyphis*, exhibits IOT when it navigates by the pattern of polarized light in the sky, but shows no IOT when it uses landmarks for orientation. This marked difference in behavioural performance may help to elucidate the neural strategies adopted by insects when using celestial (polarization) and terrestrial cues for navigation.

Insects possess multi-faceted compound eyes which endow them with full panoramic vision^{13,14}. The present experiments used the long-legged Saharan desert ant, *Cataglyphis fortis*, in which the visual fields of the two eyes cover 93% of the unit sphere¹⁵. As the remaining 7% of 'dead space' is confined completely to parts of the unit sphere that lie below the horizon, desert ants can view simultaneously all parts of the celestial hemisphere as well as the entire skyline extending above the horizon. Ants use visual skymarks such as the Sun and the extensive pattern of polarization as directional cues¹⁶. A desert ant can easily be trained to find an artificial food source established, say, 20 m to the north of its underground colony¹⁷. Having arrived there, the ant will grasp the piece of bait (say, a small cube of cheese) with its mandibles and run home along a straight path, selecting the proper home direction (in this case south) even if the insect is displaced to unknown territory and prevented from viewing either landmarks or the Sun; the celestial pattern of polarization suffices as a compass cue¹⁷.

To test IOT, we trained ants after covering one of the compound eyes (and its three ocelli) with a light-tight cap. Thus, these ants had to learn their home direction using only one eye (the 'trained eye'). After training was completed (Fig. 1, test A), we removed the cap from the 'naive eye' and occluded the other (trained) eye. The eye caps could be removed carefully using fine forceps, without damaging the corneal surfaces of the eyes. In scanning micrographs, eyes from which caps had been removed could not be distinguished from untreated eyes. Ants with the eye caps exchanged oriented in the homeward direction just as the controls did using their trained eyes (Fig. 1, test B1).

In these tests, the ants used both eyes to navigate until we started to train them to an artificial food source; only then was one eye occluded. In the following experiment, one eye was covered before the ant could acquire any visual information. This was done by rearing the ants in a laboratory colony and providing them with eye caps shortly after they had hatched from their pupal cases. The monocular ants were then transferred to a natural colony in the desert, where they remained underground until they began their foraging lives about 28 days later¹⁸. These animals were still able to perform IOT when the trained eye was covered and the naive eye opened for the first time in the ant's life, using only the pattern of polarized light in the sky (Fig. 1, test B2).

In contrast, if the ants used landmarks to pinpoint the position of their nest, IOT did not occur. This was demonstrated by marking the position of the nest by a set of artificial landmarks, consisting of three black cylinders (11° high and 6° wide), which were positioned at the corners of an equilateral triangle centred on the nest entrance (Fig. 2a). Ants were tested which had already returned from the food source to the nest; such ants have reset their path integration (celestial navigation) system to zero and rely exclusively on landmarks for finally locating the

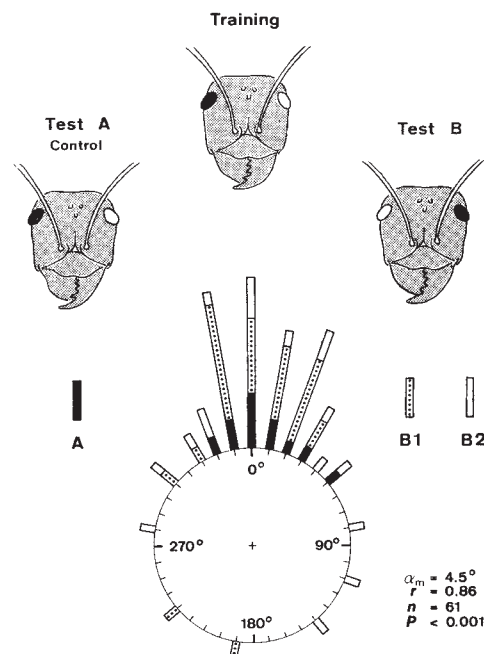


Fig. 1 Ants exhibit interocular transfer of visual information deduced from the pattern of polarized light in the sky. The bars indicate walking directions of monocular ants which can see only the pattern of polarized light in the sky. Landmarks and the Sun were occluded¹⁷. One eye was covered with a thin coat of fast-drying paint (Testors Matte Enamel 1149 Black) applied while the ant was immobilized, its head viewed through a dissecting microscope. 0°, Homeward direction. A, Control experiment: trained eye open; $n = 11$. B, Tests for interocular transfer: naive eye open. The naive eye was occluded while training the ant to the 0° compass course (B1; $n = 32$) or during the entire lifetime of the ant before the experiment (B2; $n = 18$). α_m and r are the direction and length of mean orientation vector, respectively²⁵. If one eye is occluded, ants, as well as other arthropods, deviate by an error angle towards the seeing side. In *C. fortis*, this error angle, ϵ , was determined before the experiment ($\epsilon = 37.2 \pm 4.3^\circ$ (s.e.), $n = 50$) and then used to correct the ants' compass bearings. In all tests, homeward orientation was highly significant ($P < 0.001$; Rayleigh test²⁵).

position of their nest¹⁶. The distance between the nest entrance and each cylinder was 2 m.

Monocular ants learned the configuration of landmarks while performing four to six orientation runs which led them to a distance of up to 3 m from the nest; only then did they begin foraging up to distances of more than 50 m. In the critical test, ants and landmarks were transferred to a nearby plain which had been provided with a grid of white lines to facilitate the recording of the ant's walking trajectories¹⁷. When the ants captured directly at the nest entrance were released anywhere near the triangle of cylinders, they immediately headed for the centre of the triangle and consistently searched there by walking in narrow loops around the point where they expected the nest to be (Fig. 2b, c). In contrast, when the trained eye was occluded and the naive eye opened, the ant was unable to locate the centre of the triangle to which it had been trained, but searched around the point where it was released (Fig. 2d). However, if the eye cap was immediately exchanged, leaving the trained eye open and the naive eye occluded, the ant again oriented perfectly by searching the centre of the triangle (Fig. 2b, c). Even when eye caps were changed from eye to eye several times, the same result was always obtained; the ant oriented perfectly when using the trained eye, but was disoriented completely when using the naive eye.

It may generally be assumed that IOT for some function occurs when the locus of that function is downstream from the place where information from the two eyes is combined. Lack of IOT would indicate some contribution from monocular mechanisms peripheral to binocular convergence. The landmark experiments indicate that the way in which the image of the

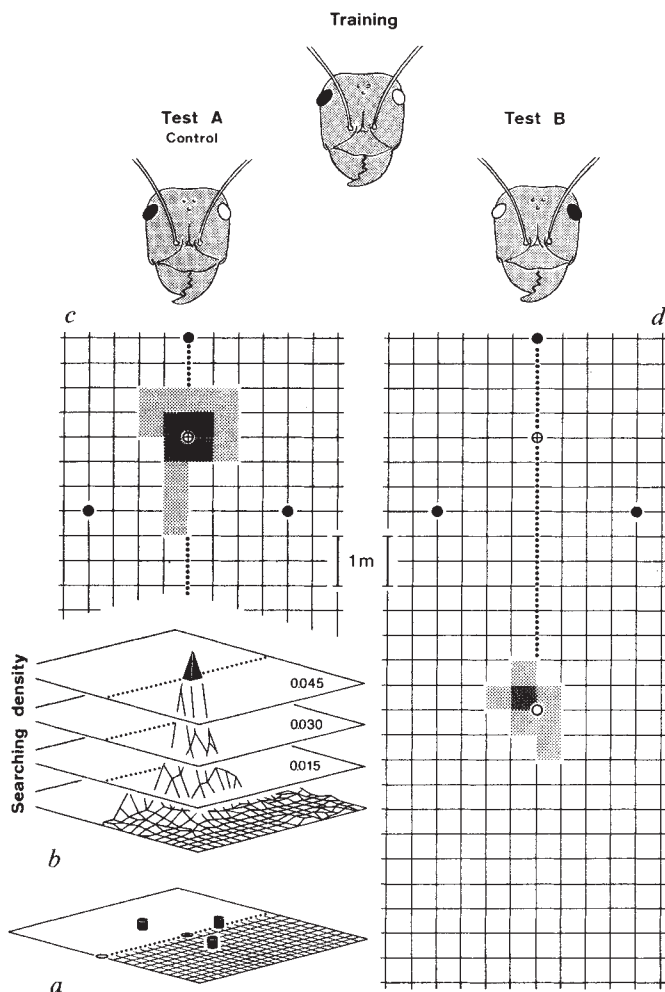


Fig. 2 Ants do not exhibit interocular transfer of visual information deduced from landmarks. *a*, Landmark configuration. Monocular ants were trained to the centre $\oplus$ of the triangle marked by three black cylinders (11° high, 6° wide as seen from the centre of the triangle). In the tests following the training procedure, the ants were released outside the triangle (at $\circ$). *b*, *c*, Test A, control experiment; trained eye open. Searching densities (*SD*) are shown in three-dimensional (*b*) and two-dimensional (*c*) plots. They were determined as follows. In the field, the ants' paths were recorded at reduced scale by using a grid of white lines painted on the hard desert ground. Later, for each of the sampling squares, the total pathlengths of all ants tested were determined by using an electronic digitizer, and the relative pathlengths per sampling square (searching densities) were computed. In *c*, *d*, the shaded areas are those that were searched by the ants most extensively: $SD > 0.045$, $0.045 > SD > 0.03$, $0.03 > SD > 0.015$. *d*, Test B, test for interocular transfer; naive eye open. The ants no longer recognized the configuration of landmarks. They searched about the point of release, but not in the centre of the triangle. In total, 30 ants were tested for 5 min each.

skyline is stored within the ant's visual system depends crucially on the information gained by each eye. A monocular ant, while turning around and scanning the entire skyline with one of its eyes, does not use the landmark information it obtains to form a central 'binocular' image that could be read and used by the other eye as well. This is consistent with the recent view that insects use internally stored images of landmarks to guide them home^{19,20}. In this context, it is interesting that birds which exhibit IOT when discriminating between shapes and colours⁷⁻⁹ also completely lack IOT when visual spatial memories are involved in the task to be solved, for example, when food sources must be located on the basis of landmark information²¹.

On the other hand, a monocular ant can read a learned compass direction from the pattern of polarized light in the sky irrespective of whether it uses the trained eye or the naive eye. In the latter case, this might be explained very simply. In bees,

and probably also in ants, the ability to use polarized sky patterns as a compass is restricted to a small specialized part of the retina located at the uppermost dorsal margin of the insect's eye²². According to our current understanding of how this specialized area is used as a celestial compass^{23,24}, the compass information read from the sky by one eye is identical to that read by the other eye. It seems that one eye can substitute for the other when the areas of the two eyes involved convey identical messages to the nervous system. In these experiments, IOT has been inferred from unilateral stimulation by light²⁶, but as the contralateral eye was not occluded, one cannot dismiss the possibility that the response was due to stimulation of the rather large binocular field of view or to stray light artefacts.

This work was supported by Swiss NSF grant 3.073.081.

Received 15 October 1984; accepted 5 March 1985.

1. Rensch, B. *Gedächtnis, Begriffsbildung und Planhandlung bei Tieren* (Parey, Berlin, 1973).
2. Hamilton, C. R. in *Analysis of Visual Behavior* (eds Ingle, D. J., Goodale, M. A. & Mansfield, R. J. W.) 693-717 (MIT Press, 1982).
3. Berlucchi, G. in *Changing Concepts of the Nervous System* (eds Strick, P. L. & Morrison, A. R.) 321-336 (Academic, London, 1982).
4. Robinson, S. R. *Brain Behav. Evol.* **21**, 114-124 (1982).
5. Pito, M. & Lepore, F. *Nature* **301**, 513-515 (1983).
6. Mohn, G. & Steele Russell, I. *Behav. Brain Res.* **7**, 371-378 (1983).
7. Goodale, M. A. & Graves, J. A. in *Analysis of Visual Behavior* (eds Ingle, D. J., Goodale, M. A. & Mansfield, R. J. W.) 211-240 (MIT Press, 1982).
8. Mihara, M. & Watanabe, S. *Behav. Brain Res.* **4**, 411-416 (1982).
9. Mallin, H. D. & Delius, J. D. *Behav. Anal. Lett.* **3**, 297-309 (1983).
10. Schulte, A. *Z. vergl. Physiol.* **39**, 432-476 (1957).
11. Ingle, D. *Brain Behav. Evol.* **1**, 58-85 (1968).
12. Muntz, W. R. A. *J. comp. Physiol. Psychol.* **54**, 49-55 (1961).
13. Stavenga, D. G. in *Handbook of Sensory Physiology* Vol. 7, Pt 6a (ed. Autrum, H.) 357-439 (Springer, Berlin, 1979).
14. Wehner, R. & Srinivasan, M. in *Insect Communication* (ed. Lewis, T.) 29-47 (Academic, London, 1984).
15. Wehner, R. *Senckenberg. biol.* **64**, 89-132 (1983).
16. Wehner, R. *A. Rev. Ent.* **29**, 277-298 (1984).
17. Wehner, R. *NeuJbL naturf. Ges. Zürich* **184**, 1-132 (1982).
18. Schmid-Hempel, P. thesis, Univ. Zurich (1983).
19. Cartwright, B. A. & Collett, T. S. *J. comp. Physiol.* **151**, 521-543 (1983).
20. Wehner, R. in *Neuroethology and Behavioral Physiology* (eds Huber, F. & Markl, H.) 366-381 (Springer, Berlin, 1983).
21. Sherry, D. F., Krebs, J. R. & Cowie, R. J. *Anim. Behav.* **29**, 1260-1266 (1981).
22. Wehner, R. & Strasser, S. *Physiol. Ent.* (in the press).
23. Rossel, S. & Wehner, R. *J. comp. Physiol.* **A154**, 607-615 (1984).
24. Wehner, R. & Rossel, S. in *Experimental Behavioral Ecology and Sociobiology* (eds Hoelldobler, B. & Lindauer, M.) 11-53 (Fischer, Stuttgart, 1985).
25. Batschelet, E. *Circular Statistics in Biology* (Academic, London, 1981).
26. Masuhr, T. & Menzel, R. in *Information Processing in the Visual Systems of Arthropods* (ed. Wehner, R.) 315-321 (Springer, Berlin, 1972).

Voltage-dependent potassium currents in cultured astrocytes

Stuart Bevan & Martin Raff

Zoology Department, University College London,
Gower Street, London WC1E 6BT, UK

Astrocytes are a major cell type in the mammalian central nervous system (CNS), yet their functions remain uncertain. There are two principal classes of these glial cells—protoplasmic astrocytes, found mainly in grey matter, and fibrous astrocytes, which occur mainly in white matter¹. Recently, these two types of astrocytes have been distinguished in cultures of developing CNS and have been shown to be biochemically distinct². Because both types contain large numbers of glial filaments *in vitro* and hence appear 'fibrous', we will refer to them as type 1 (protoplasmic) and type 2 (fibrous) astrocytes². Most type 2 astrocytes in culture share several properties with neurones²; for example, they have a process-bearing morphology and bind tetanus toxin and the monoclonal antibody A2B5 (ref. 3), both of which recognize specific gangliosides^{3,4} and were initially considered to be neurone-specific markers in the CNS^{3,5,6}. We have therefore investigated whether type 2 astrocytes also share electrophysiological properties with neurones. Using intracellular microelectrode and 'whole-cell' (patch-clamp) recording techniques, we have now found that both type 1 and type 2 astrocytes in culture have time- and voltage-dependent potassium ion conductances which, until recently, were considered to be confined largely to electrically excitable cells.

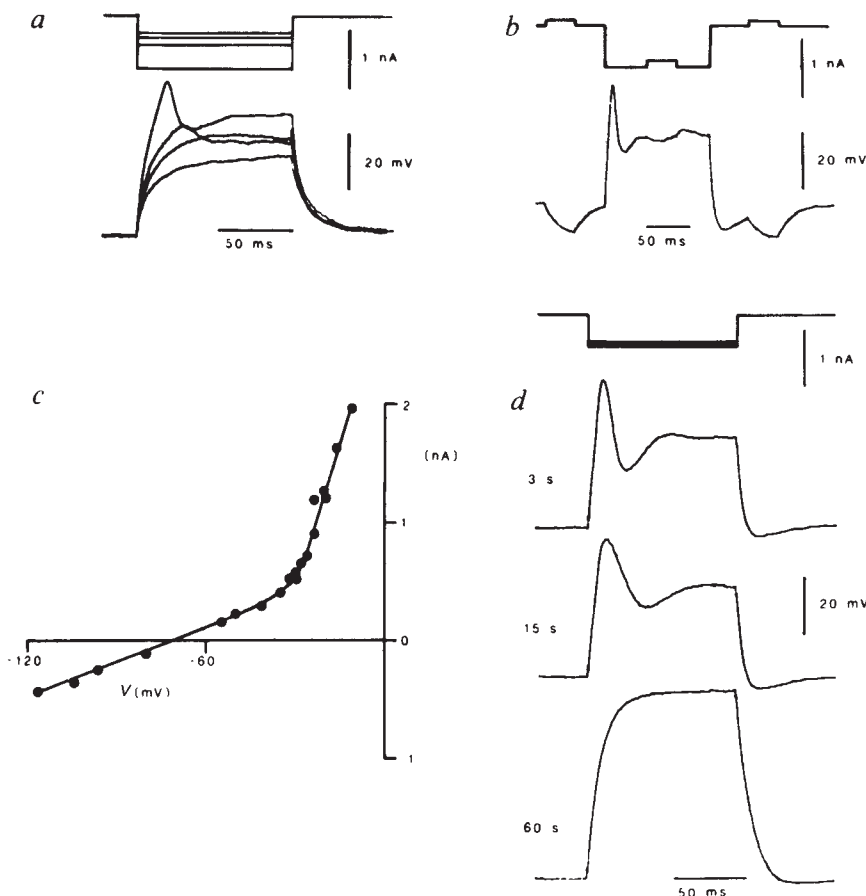


Fig. 1 *a*, Voltage response (lower trace) of a type 2 astrocyte to depolarizing current pulses of varying amplitude (upper trace). Resting potential, -78 mV. *b*, Hyperpolarizing current pulses delivered before, during and after a depolarizing pulse (upper trace) show that the membrane conductance is increased during the plateau phase of the voltage response (lower trace). Resting potential, -75 mV. *c*, Current-voltage ($I-V$) plot for depolarizing and hyperpolarizing current pulses shows an increase in membrane conductance for depolarizations to >-40 mV. The membrane potential was measured at the end of the current pulse, on the plateau phase of the voltage response. *d*, Membrane responses to depolarizing current pulses injected from a 2 M CsCl-filled intracellular microelectrode at the specified times after electrode impalement. Note the initial complex nature of the depolarizing response and its disappearance with time. The current pulse for the middle voltage trace is slightly smaller than for the other two traces. Resting potential, -80 mV. Except where noted, intracellular microelectrode studies were made on cells bathed in a recording solution of (mM): 145 NaCl, 5 KCl, 10 CaCl₂, 5 HEPES-NaOH pH 7.4. The use of a high calcium concentration improved the quality of electrode impalement and gave more negative and more stable membrane potentials. Microelectrodes of resistance 30–45 M Ω were filled with 3 M KCl except in *d*. Current pulses were delivered through the recording electrode which was connected to a bridge circuit. Temperature, $19-21^{\circ}\text{C}$.

Cells from 7-day-old Sprague-Dawley rat optic nerves were dissociated and cultured on poly-L-lysine-coated plastic culture dishes in Dulbecco's modified Eagle's medium containing 10% fetal calf serum (FCS) as previously described^{2,7}. We have previously shown that there are no neurofilament-containing neurones in such cultures² and that $>90\%$ of the process-bearing cells are type 2 astrocytes, as determined by labelling with A2B5 antibody and antibodies against glial fibrillary acidic protein (GFAP)⁸. The remaining process-bearing cells are oligodendrocytes, as determined by their labelling with antibody against galactocerebroside (GC)^{8,9}.

After 2–5 days in culture, cells with a typical type 2 astrocyte morphology² were examined electrophysiologically. Resting membrane potentials, measured with an intracellular microelectrode, were in the range -65 to -85 mV (mean $\pm$ s.d. = -79.6 ± 5.9 mV; $n=29$). When the cells were polarized by injecting current pulses, a 'passive' membrane response was noted for small depolarizing or hyperpolarizing responses; however, with larger depolarizing pulses the response was more complex and a delayed reduction in the size of the depolarization was observed (Fig. 1*a*). This decline in amplitude to a plateau level was associated with an increase in membrane conductance, which was detected in two ways. First, when small hyperpolarizing current pulses were delivered before, during and after the depolarization (Fig. 1*b*), the evoked hyperpolarization was reduced during the plateau phase of the response. Second, when the relationship between the amplitudes of the injected current and the membrane potential near the end of the pulse was examined, a clear increase in membrane conductance was noted with depolarizations beyond -40 mV (Fig. 1*c*).

This voltage-dependent response was not affected by removal of external Na⁺ (by replacement with *N*-methyl-D-glucamine), by addition of tetrodotoxin (TTX, 10 nM), by reducing the external Ca²⁺ level to 10^{-8} M with added EGTA or by addition of the Ca²⁺-channel blocking agents La³⁺ (1 mM), Cd²⁺ (10 mM) and nifedipine (1 μ M) to a nominally 'calcium-free' solution. In contrast, the response was attenuated by addition

of the K⁺-channel blocking drugs, tetraethylammonium (TEA, 10 mM) and 4-aminopyridine (4-AP, 5 mM). Furthermore, the voltage-activated increase in membrane conductance was greatly reduced when cells were impaled with microelectrodes containing Cs⁺, which blocks K⁺ channels in many cell types^{10,11}. Figure 1*d* shows the response of a type 2 astrocyte to similar current pulses delivered 3, 15 and 60 s after penetration with a CsCl-filled microelectrode; a reduced voltage- and time-dependent response occurred as Cs diffused into the cell from the pipette. These findings suggest that the channels mediating the response to depolarization are similar to voltage-gated K⁺ channels found in neurones and muscle cells.

To investigate this voltage-dependent response further, type 2 astrocytes were studied by the patch-clamp method with 'whole-cell' voltage-clamp recordings¹². The measured resting potential, with 145 mM KCl in the patch pipette ($[\text{K}^+]_o = 5$ mM), was -80.1 ± 3.5 mV (mean $\pm$ s.d., $n=21$), which is close to the value calculated from the Nernst equation for an exclusively K⁺-permeable membrane. Figure 2*a* shows a family of voltage-clamp currents evoked by depolarizing voltage steps from a holding potential of -80 mV. After the initial capacity current, time- and voltage-dependent outward currents occurred with depolarizations to >-40 mV. This is shown graphically in Fig. 2*b*, where the current amplitude at the end of the depolarizing pulse is plotted against membrane potential. The voltage-dependent response was altered when Cs⁺ (10 mM), TEA (10 mM) or 4-AP (2 mM) was included in the internal solution of the patch pipette; depolarizing steps now no longer evoked a persistent outward current. However, with Cs⁺ present, an outward current was observed which peaked within a few milliseconds and then rapidly became inactivated, declining exponentially to a steady level with a time constant of ~ 30 ms (Fig. 2*c*). Unlike the persistent outward current, this transient current was abolished if the holding potential was set to levels >-40 mV. Because, in our experimental conditions, this current is outward at negative membrane potentials, it also must be carried largely by K⁺ ions.

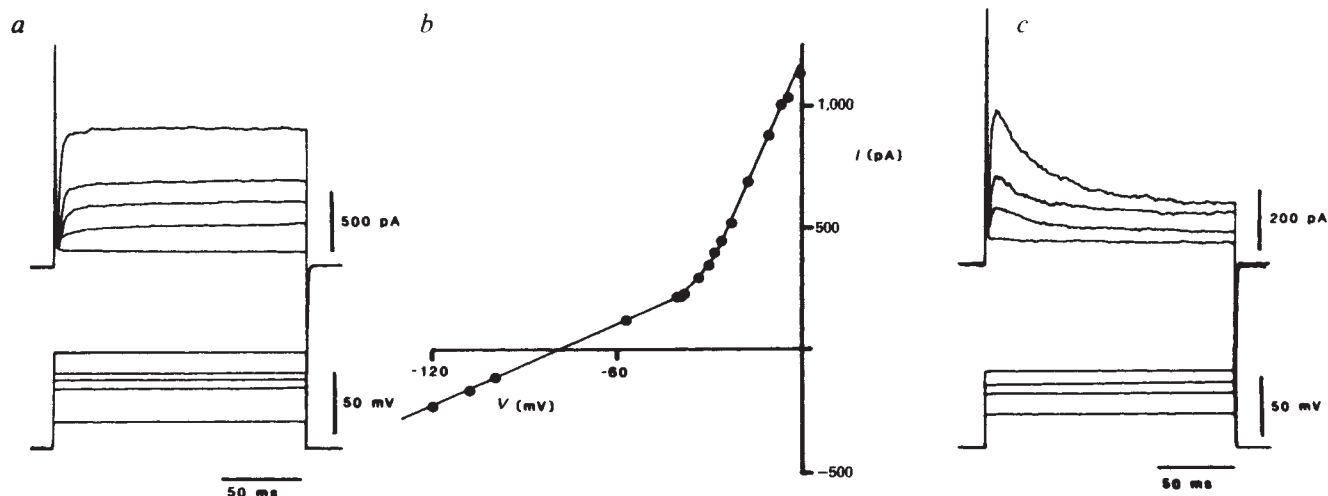


Fig. 2 Voltage clamp studies on type 2 astrocytes made with the whole cell (patch clamp) recording method. *a, c*, Outward currents (upper traces) evoked by depolarizing voltage command steps (lower traces) from a holding potential of -80 mV; time-dependent currents are evoked by depolarizations to >-40 mV. Cell resting potentials: *a*, -80 mV; *b*, -83 mV. In *c* the solution in the patch pipette was supplemented with 10 mM CsCl₂. *b*, *I-V* plot of 'steady-state' current for the cell shown in *a*. For these studies, cells were bathed in a recording solution containing 2 mM CaCl₂. Patch pipettes (3–8 M Ω resistance) were filled with (mM): 145 KCl, 5 NaCl, 1 EGTA, 5 HEPES-KOH pH 7.4. TEA (10 mM), 4-AP (2 mM) or CsCl (10 mM) was added to this solution as required. Gigaohm seals (>25 G Ω) were formed between the patch pipette and the membrane surface by gentle suction; increased suction then ruptured the underlying membrane to give whole-cell recordings. No series resistance or capacity compensations were made for the illustrated records. Temperature, 20–22 °C. The complex geometry of the cells means that although voltage control of the soma is good, the long processes will be poorly clamped. However, this does not affect the basic reported features of the evoked currents, as the kinetics and reversal potentials of the whole-cell currents and of the macroscopic currents in outside-out membrane patches are indistinguishable.

To determine whether type 1 astrocytes in culture have similar voltage- and time-dependent K⁺ conductances, we examined cultures of type 1 astrocytes prepared from neonatal rat cortex by a modification of the McCarthy and de Vellis procedures¹³ described elsewhere². More than 95% of the cells in such cultures were A2B5⁺, GFAP⁺ type 1 astrocytes. Only cells that were not in contact with other cells were examined electrophysiologically, to avoid any problems introduced by electrical coupling between contiguous cells¹⁴. Type 1 astrocytes had similar resting membrane potentials to type 2 astrocytes and also showed a voltage- and time-dependent outward current on depolarization (Fig. 3*b*). Examination of the 'tail' currents seen immediately after stepping back from a depolarized level to various holding potentials (-60 to -100 mV) indicated a reversal potential of ~ -85 mV, which is consistent with these voltage-activated channels being primarily K⁺-permeable.

To determine whether cultured oligodendrocytes have similar K⁺ conductances, we examined oligodendrocytes in optic nerve cultures prepared from 7-day-old rats; the cells were grown in

a modified Bottenstein and Sato medium containing 0.5% or no FCS for 2–5 days as described previously⁸. In these cultures, more than 95% of process-bearing cells are GC⁺, GFAP⁺ oligodendrocytes⁸. Although such cells had resting membrane potentials similar to those of type 1 and type 2 astrocytes, no voltage-dependent conductances were activated when the cells were depolarized to levels up to $+20$ mV. Figure 3*a* shows the membrane response to depolarizing pulses and the current-voltage relationship for a typical oligodendrocyte. Similar results with cultured oligodendrocytes have been reported by Kettenmann *et al.*¹⁵. The electrophysiological differences between type 2 astrocytes and oligodendrocytes must appear rapidly during development, because it has been shown that both of these cells develop within 2 days *in vitro*⁷ from a common progenitor cell present in 7-day-old optic nerve⁸: in the presence of 10% FCS, the bipotential progenitor cell differentiates into a type 2 astrocyte, while in serum-free medium it differentiates into an oligodendrocyte⁸. It is not clear whether the progenitor cell itself has the voltage-dependent conductances seen in type 2 astrocytes

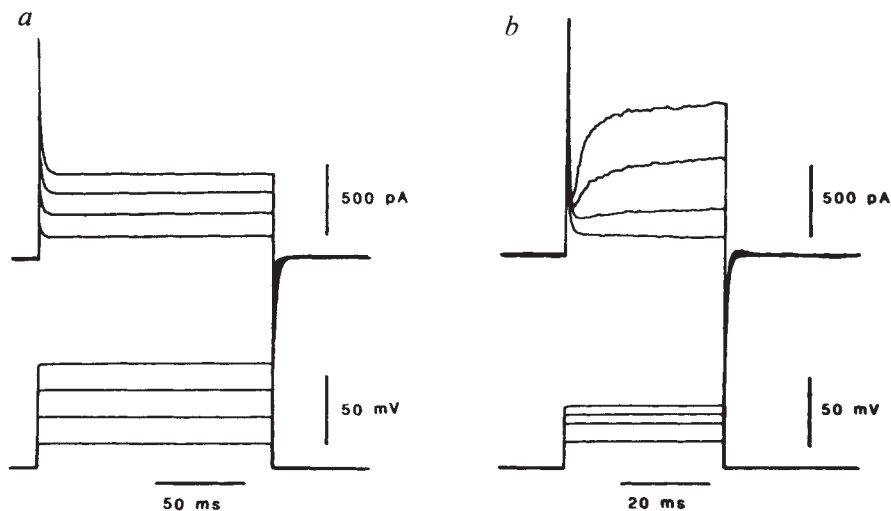


Fig. 3 Voltage clamp studies on an oligodendrocyte (*a*) and a type 1 astrocyte (*b*). Before electrophysiological examination, the flat type 1 astrocytes were usually rounded-up by a brief (~ 10 min) preincubation in a low-calcium-containing solution; this procedure eliminated most of the cell processes and thus improved the spatial control of membrane voltage. Whole-cell recordings show a voltage- and time-dependent K⁺ current for type 1 astrocytes but not for oligodendrocytes. Holding potentials, -80 mV.

and loses them when it differentiates to become an oligodendrocyte, or whether the progenitor cell acquires these conductances on becoming a type 2 astrocyte. In either case, the changes in membrane conductance properties must occur within 2 days.

Thus, we have demonstrated voltage-activated K^+ conductances in type 1 and type 2 astrocytes in culture; these were not found in oligodendrocytes. Such conductances are activated when the cell is depolarized beyond -40 mV and seem to result from the opening of K^+ channels. The outward current normally observed in type 2 astrocytes shows little or no inactivation with time (over several seconds) and is blocked by TEA, 4-AP and Cs^+ ; this conductance resembles the delayed rectifier found in nerve and muscle cells. In the presence of internal Cs^+ , a smaller, rapidly inactivating outward current is seen. The kinetics of this current may reflect a voltage-dependent block of the delayed rectifier-type channels by Cs^+ (see ref. 16) or the presence of a second type of K^+ -permeable channel in the membrane. Our findings suggest that at least some of the neural tumour cell lines reported to contain voltage-dependent K^+ channels as well as glial cell antigens¹⁷ arose from astrocytes or their precursor cells.

We have shown that astrocytes in culture display voltage-activated K^+ channels. This finding complements the recent reports of veratridine- and voltage-activated Na^+ channels^{18,19} and voltage-dependent Ca^{2+} channels²⁰ in type 1 astrocytes. The

biological significance of these conductances in astrocytes will remain uncertain until the functions of these cells are known.

M.R. is supported by a programme grant from the MRC.

Received 16 October 1984; accepted 6 March 1985.

1. Peters, A., Palay, S. L. & Webster, H. de F. *The Fine Structure of the Nervous System: The Neurons and Supporting Cells* 2nd edn, 233–244 (Saunders, Philadelphia, 1976).
2. Raff, M. C., Abney, E. R., Cohen, J., Lindsay, R. & Noble, M. J. *Neurosci.* **3**, 1289–1300 (1983).
3. Eisenbarth, G. S., Walsh, F. S. & Nirenberg, M. *Proc. natn. Acad. Sci. U.S.A.* **76**, 4913–4917 (1979).
4. Van Heyningen, W. E. *J. gen. Microbiol.* **31**, 375–387 (1963).
5. Dimpfel, W., Neale, J. H. & Habermann, E. *Naunyn-Schmiedeberg's Archs Pharmacol.* **290**, 329–333 (1975).
6. Mirsky, R., Wendon, L. M. B., Black, P., Stolkin, C. & Bray, D. *Brain Res.* **148**, 251–259 (1978).
7. Raff, M. C., Williams, B. P. A. & Miller, R. H. *EMBO J.* **3**, 1857–1864 (1984).
8. Raff, M. C., Miller, R. H. & Noble, M. *Nature* **303**, 390–396 (1983).
9. Raff, M. C. *et al. Nature* **274**, 813–816 (1978).
10. Adelman, W. J. & Senft, J. P. *J. gen. Physiol.* **50**, 279–293 (1966).
11. Benzanilla, F. & Armstrong, C. M. *J. gen. Physiol.* **60**, 588–608 (1972).
12. Marty, A. & Neher, E. in *Single Channel Recording* (eds Sakmann, B. & Neher, E.) 107–122 (Plenum, New York, 1983).
13. McCarthy, K. D. & de Vellis, J. *J. Cell Biol.* **85**, 890–902 (1980).
14. Kettenmann, H., Orkand, R. K. & Schachner, M. *J. Neurosci.* **3**, 506–516 (1983).
15. Kettenmann, H. *et al. Pflügers Arch. ges. Physiol.* **401**, 324–332 (1984).
16. Armstrong, C. M. & Hille, B. *J. gen. Physiol.* **59**, 388–400 (1972).
17. Stallcup, W., Levine, J. & Rashke, W. *Cold Spring Harb. Rep. Neurosci.* **2**, 39–49 (1981).
18. Bowman, C. L., Kimelberg, H. K., Frangakis, M. V., Berwald-Netter, Y. & Edwards, C. J. *Neurosci.* **4**, 1527–1534 (1984).
19. Bevan, S., Chiu, S. Y., Gray, P. T. A. & Ritchie, J. M. *J. Physiol., Lond.* (in the press).
20. MacVicar, B. A. *Science* **226**, 1345–1347 (1984).

Expression of T-cell antigen receptor genes during fetal development in the thymus

H. Ralph Snodgrass, Zlatko Dembić, Michael Steinmetz & Harald von Boehmer

Basel Institute for Immunology, Grenzacherstrasse 487, CH-4058 Basel, Switzerland

The T-cell antigen receptor is a heterodimeric molecule composed of α - and β -subunits of relative molecular mass 40,000–50,000 (refs 1–6). Recently, the genes encoding both the β -^{7–9} and α -^{10–12} chains have been cloned. By comparing amino-acid and nucleic-acid sequences, it is clear that these genes encode the α - and β -proteins of the T-cell receptor^{13,14}. In addition, a third receptor-like gene, the γ -chain gene, has been identified¹⁵, which has many structural and sequence characteristics in common with the α - and β -chain genes. The role of the γ -chain gene in T-cell development is unknown. We have reported recently that the β -chain genes are transcriptionally turned on in the thymus at about day 17 of fetal development¹⁶. Here we report that the α -chain also is transcriptionally activated during this time, but that the γ -chain gene is active in the thymus at day 14, reaches a peak steady-state level at day 15 and rapidly declines thereafter. If the γ -chain gene has a functional role, it would seem to be involved very early in T-cell development, before the mature T-cell receptor is expressed.

The mouse thymus is initially colonized by blood-borne precursors during days 11 and 12 of gestation¹⁷. Cell-surface molecules with the characteristics of the T-cell antigen receptor do not appear until day 17 (ref. 16) and functional cells do not appear in appreciable numbers until day 19 (refs 18, 19). Consistent with this, the α and β loci are transcriptionally active around day 17 of gestation (Fig. 1); this is evident not only in the absolute levels of messenger RNA, but also in the amounts of the larger transcripts (1.6, 1.3 and 1.5 kilobases (kb) for α , β and γ , respectively) relative to those of the smaller transcripts (1.3, 1.0 and 1.2 kb for α , β and γ , respectively). This is important because in the case of the β -chain gene, the larger transcript represents the full-length functional mRNA, whereas the smaller mRNA is an aberrant transcript representing only rearrangements of the diversity-joining-constant gene regions^{20,21}. As

reported previously^{16,22}, the levels of β -chain mRNA reach a peak in the adult thymus and drop substantially in peripheral T cells, in contrast to steady-state levels of α -chain mRNA, which reach a maximum by day 19 and remain high in peripheral T cells.

From the data presented in Fig. 1, it is impossible to compare the relative amounts of α , β and γ mRNA because of the different sizes of probes used and the different exposure times. A more quantitative estimate of the relative levels of expression is obtained from the number of clones of each of these genes present in a complementary DNA library. A library of 5×10^4 independent cDNA clones was prepared from day 17 fetal thymocytes, containing 50 (0.1%) β -chain clones and 5 (0.01%) clones each of the α - and γ -chains. (In a similar experiment, Chien *et al.*¹⁰ found frequencies of 0.01% (α), 0.2% (β) and 0.002% (γ) in the adult thymus and 0.03% (α), 0.1% (β) and 0.002% (γ) in peripheral T cells.) These differences in expression between day 17, adult thymus and peripheral T cells are reflected in the hybridization intensities shown in Fig. 1. The inverse relationship in the expression of the α - and γ -chain genes is striking. The γ -chain gene is transcribed at high levels early in differentiation and then transcription declines rapidly at the time when the α -chain gene is becoming activated.

These data further support our previous conclusions¹⁶ that the mature receptor seen on peripheral T cells is first expressed in the thymus at ~day 17 of gestation. The role of the γ -chain gene is unknown. Our data are consistent with a change in receptor repertoire during ontogeny, as suggested in some models^{23,24}. One speculation, consistent with the expression of the α -, β - and γ -chain genes during ontogeny, is that the β - and γ -proteins form receptors that have specificity for major histocompatibility complex (MHC) antigens either alone or in combination with a ubiquitous cell-surface antigen; in other words, perhaps the β/γ combination is a MHC-restricted receptor specific for a nonpolymorphic cell-surface determinant. Thymocytes with the β/γ combination specific for self-MHC antigens are induced to differentiate. One of the differentiation steps would include the turning-off of the γ -chain gene and turning-on of the α -chain gene. The new α/β receptor would now (or perhaps after another round of selection) have specificity for a wide range of non-MHC antigens and for self-MHC. The binding site for self-MHC would be contributed by the β -chain, whereas the binding site for non-MHC would be formed by both the α - and β -chains. Although this, in effect, suggests the

presence of two binding sites, the overall functional specificity would depend on the specific α/β combination. Such a model is compatible with what is known about the structure of the β -chain variable region²⁵, as well as the extent of variability of the other two genes^{10,11,15}. This would explain the lack of observed mixing of specificities in somatic cell hybrids (ref. 26 and W. Haas, personal communication). There is no direct evidence either for or against such a model. More information is needed concerning the expression of the γ -chain on the cell surface and the repertoire of the three genes before and after thymic selection.

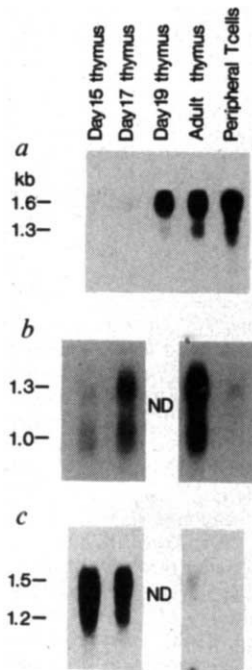


Fig. 1 Northern blot analysis of T cells at various stages of development. Total RNA (20 μ g) was separated on 1.2% glyoxal gels²⁷, transferred to Gene Screen (NEN) and hybridized with an α -chain probe (a), β -chain probe (b), or γ -chain probe (c). b, From ref. 16; ND, not done.

Methods. B10.BR fetal thymocytes were obtained from timed matings where the day of vaginal plug detection was designated as day 0. RNA was isolated by the guanidinium isothiocyanate and CsCl gradient centrifugation procedures^{28,29}. RNA blots were hybridized in 1 M NaCl, 50 mM Tris pH 7.5, 1% SDS, 0.2% polyvinylpyrrolidone, 0.2% bovine serum albumin, 0.2% Ficoll, 100 μ g ml⁻¹ denatured salmon sperm DNA, 10% dextran sulphate and 1.5 $\times 10^6$ c.p.m. ml⁻¹ oligo-labelled³⁰ probe (10⁹ c.p.m. μ g⁻¹) at 68 °C overnight. Filters were washed in 0.3 M NaCl, 60 mM Tris pH 8, 2 mM EDTA twice at 25 °C for 10 min and twice at 68 °C for 30 min, and twice in 10 mM NaCl, 6 mM Tris pH 8, 2 mM EDTA at 25 °C. Exposure was overnight with intensifying screens (Ilford Fast Tungstate). Sizes of RNA molecules were estimated from denatured phage DNA restriction fragments of known lengths run in parallel on the same gel. All probes are gel-purified inserts. The α -chain probe is the 300-base pair (bp) *NcoI/AvaII* fragment from the constant portion of the α -chain cDNA clone T1.2 (ref. 12). The β -probe is the 300-bp insert of the 4.4-kb cDNA clone from the constant portion of the β -chain gene starting ~50 bp downstream of the joining (*J*) gene segment (M. Kiefer and M.S., unpublished results). This clone was isolated together with the 4.1-kb β -chain cDNA clone, as described previously¹⁶. The γ -chain probe represents the 1.3-kb insert of the T γ 5 cDNA clone containing an almost full-length γ -chain cDNA as determined by comparison of this clone with published¹⁵ γ -chain cDNA clones. The T γ 5 cDNA clone was isolated from an EL-4 cDNA library with a 33-residue oligonucleotide specific for the constant (*C γ*) gene segment (M.S., W. Bannwarth and M. Kiefer, unpublished results). This cDNA clone has a 105-bp deletion starting at amino acid -4 and extending to amino acid 32 (numbered according to ref. 15; A. Traunecker, personal communication), presumably a result of aberrant splicing.

We thank Carina Olsson, Katrin Schubiger and Gholam Das-toornikoo for technical assistance; Wilhelm Bannwarth for synthesizing the oligonucleotide probes; Lynn Gisler for preparing the manuscript; and Werner Haas and Pawel Kisielow for helpful discussions. The Basel Institute for Immunology is supported by F. Hoffmann-La Roche and Co., Basel, Switzerland.

Note added in proof: Since the submission of this manuscript, Raulet and co-workers³¹ have reported results consistent with the data presented here.

Received 17 January; accepted 11 March 1985.

- Allison, J., McIntyre, B. & Block, D. *J. Immun.* **129**, 2293-2300 (1982).
- Meuer, S. *et al. J. exp. Med.* **157**, 705-719 (1983).
- Haskins, K. *et al. J. exp. Med.* **157**, 1149-1169 (1983).
- Acuto, O. *et al. J. exp. Med.* **158**, 1368-1373 (1983).
- McIntyre, B. & Allison, J. *Cell* **34**, 739-746 (1983).
- Kappler, J. *et al. Cell* **35**, 295-302 (1983).
- Hedrick, S., Cohen, D., Nielson, J. & Davis, M. *Nature* **308**, 149-153 (1984).
- Hedrick, S., Nielsen, E., Kavaler, J., Cohen, D. & Davis, M. *Nature* **308**, 153-158 (1984).
- Yanagi, Y. *et al. Nature* **308**, 145-149 (1984).
- Chien, Y. *et al. Nature* **312**, 31-35 (1984).
- Saito, H. *et al. Nature* **312**, 36-40 (1984).
- Dembić, Z., Bannwarth, W., Taylor, B. & Steinmetz, M. *Nature* **314**, 271-273 (1985).
- Acuto, O. *et al. Proc. natn. Acad. Sci. U.S.A.* **81**, 3851-3855 (1984).
- Sim, G. *et al. Nature* **312**, 771-775 (1984).
- Saito, H. *et al. Nature* **309**, 757-762 (1984).
- Snodgrass, H. R., Kisielow, P., Kiefer, M., Steinmetz, M. & von Boehmer, N. *Nature* **313**, 592-595 (1985).
- Moore, M. & Owen, J. *J. exp. Med.* **126**, 715-725 (1967).
- Widmer, M., MacDonald, H. & Cerottini, J. *Thymus* **2**, 245-255 (1981).
- Ceredig, R., Dialynas, D., Fitch, F. & MacDonald, H. *J. exp. Med.* **158**, 1654-1671 (1983).
- Clark, S. *et al. Nature* **311**, 387-389 (1984).
- Siu, J. *et al. Nature* **311**, 344-350 (1984).
- Yoshikai, Y., Yanagi, Y., Suciu-Foca, N. & Mak, T. *Nature* **310**, 506-508 (1984).
- Jerne, N. *Eur. J. Immun.* **1**, 1-9 (1971).
- von Boehmer, H., Haas, W. & Jerne, N. *Proc. natn. Acad. Sci. U.S.A.* **75**, 2439-2442 (1978).
- Patten, P. *et al. Nature* **312**, 40-46 (1984).
- Kappler, J., Skidmore, B., White, J. & Marrack, P. *J. exp. Med.* **153**, 1198-1214 (1981).
- McMaster, G. & Carmichael, J. *Proc. natn. Acad. Sci. U.S.A.* **74**, 4835-4838 (1977).
- Chirgwin, J., Prybyla, A., MacDonald, R. & Rutter, W. *Biochemistry* **18**, 5294-5299 (1979).
- Glisin, V., Crkvenjakow, R. & Byus, C. *Biochemistry* **13**, 2633-2637 (1977).
- Feinberg, A. & Vogelstein, B. *Analyt. Biochem.* **132**, 6-13 (1983).
- Raulet, D., Garman, R., Saito, H. & Tonegawa, S. *Nature* **314**, 103-107 (1985).

Interleukin-2 stimulates association of protein kinase C with plasma membrane

William L. Farrar* & Wayne B. Anderson†

* Laboratory of Molecular Immunoregulation, National Cancer Institute-Frederick Cancer Research Facility, Frederick, Maryland 21701, USA

† Laboratory of Tumor Immunology and Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205, USA

Interleukin-2 (IL-2) is a regulatory peptide important for the growth and differentiation of antigen-specific T lymphocytes and large granular lymphocytes^{1,2}. Interaction of IL-2 with its specific receptor results in the promotion of S-phase progression as well as, in certain circumstances, the production and release of γ -interferon (IFN- γ)³⁻⁵. Although the binding of IL-2 with high-affinity specific receptors has been well characterized^{6,7}, the intracellular mechanisms by which this ligand-receptor interaction promotes growth and differentiation are unknown. Here, we present evidence that IL-2/receptor interaction produces a rapid and transient redistribution of protein kinase C (PK-C) from the cytosol to the plasma membrane. Phorbol myristate acetate (PMA) also induces PK-C transposition in an analogous manner, except that PMA-induced PK-C transposition to the plasma membrane is apparently protracted. As phorbol esters have been shown to mimic IL-2 in the regulation of cellular proliferation as well as IFN- γ production⁸⁻¹⁰, the activation of PK-C by either phorbol esters or IL-2/receptor interaction seems to have a crucial role in signal transduction elicited by these extracellular messengers.

Figure 1 shows the time course of IL-2-induced protein kinase C activity. IL-2-dependent murine CT6 cells grown in 5 units of IL-2 per 10⁵ cells were washed in serum-free medium three times, 24 h before experimental stimulation. The cells were

washed twice more, resuspended to 1×10^7 cells ml^{-1} , and incubated at 37°C for 2 h. Approximately 500 units of recombinant IL-2 were added per 10^7 cells and the PK-C-specific activity in the cytosol and plasma membrane were compared at the indicated time intervals. Within 3 min of IL-2 stimulation, an increase in PK-C-specific activity of over fourfold was observed in the plasma membranes of CT6 cells compared with unstimulated controls. Increases in membrane-associated PK-C activity of over 10-fold were observed by 10 min after IL-2 stimulation, followed by a rapid decline of plasma membrane PK-C activity at 20 min and a return to initial PK-C membrane-specific activity by 60 min. Concomitant with the rapid increases of PK-C membrane-associated activity, there was a precipitous decline in PK-C activity within the corresponding cytosol preparations obtained at the same time intervals. Although a decline in cytosolic PK-C activity was seen as early as 1 min after IL-2 stimulation, the lowest PK-C cytosolic activity was observed at 10 min, corresponding to peak plasma membrane-associated activity. Cytosolic PK-C activity progressively increased to basal levels by 60 min in the continuous presence of IL-2. In the same experiment, we compared the effects of PMA with those of IL-2. As reported previously¹¹, PMA (10 ng per 10^6 cells) stimulated a rapid (1 min) association of PK-C with particulate membranes. The PMA-stimulated membrane association of PK-C was approximately twofold greater than that stimulated by IL-2 and exhibited a more protracted dissociation from CT6 plasma membranes. CT6 cytosolic PK-C activity rapidly declined within 1 min of phorbol ester stimulation and was depressed to 50–115 pmol throughout the experiment. Similar data were obtained in a previous study in which murine thymoma EL4 cells were stimulated with phorbol esters¹¹. The present results indicate that the decrease in cytosolic PK-C activity and consequent membrane association is not limited to phorbol ester-treated EL4 cells or parietal yolk sac (PYS-2) cells^{11,12}. Indeed, IL-2, a growth-stimulating peptide, induced a substantial decrease in cytosolic PK-C activity with a concomitant increase in the plasma membrane-associated activity (Fig. 1). Attempts to dissociate PK-C from the membrane fraction of PMA- or IL-2-treated CT6 cells with high-salt (0.3 M NaCl)-Tris buffer or with buffer containing metal chelators (1 mM EDTA and EGTA) have been unsuccessful (data not shown), suggesting that PK-C has become a tightly associated integral membrane component.

To further investigate whether there is a direct correlation between IL-2-induced PK-C membrane association and IL-2-induced stimulation of proliferation of CT6 cells, we compared the dose-response curve for PK-C membrane association with that for IL-2-induced proliferation. Figure 2 shows the results of a typical experiment comparing the effects of recombinant IL-2 on PK-C plasma membrane association and CT6 proliferation; the titration curves have similar slopes. Concomitant with the increased membrane association of PK-C, there was a decrease in cytosol-specific activity, with half-maximal translocation of kinase activity in both membrane and cytosol occurring within an equivalent range of IL-2 activity (65–85 units). These dose-response kinetics further reinforce the notion that cytosolic PK-C became associated with the plasma membrane. In a related study, crude homogenates were prepared from control, IL-2- or PMA-stimulated CT6 cells and the total homogenate was extracted (cytosol plus membrane) with Nonidet P-40 (NP40). The resulting detergent-solubilized, DEAE-purified total PK-C activity from either control, IL-2- or PMA-stimulated CT6 cells agreed within $\pm 15\%$, indicating that neither IL-2 nor PMA significantly altered total enzyme activity (data not shown). Also, the 50% effective dose (ED_{50}) of IL-2 for both PK-C membrane association and IL-2 bioactivity (measured by the proliferative response of CT6 cells) titrated within the same range of IL-2 concentration (80–145 units), suggesting a close correlation between the activation of PK-C membrane association and those events involved in IL-2-induced proliferation of CT6 cells.

Phospholipid enzymatic degradation, particularly phosphatidylinositol and phosphatidylcholine turnover stimulated by a wide variety of neurotransmitters, hormones, growth factors

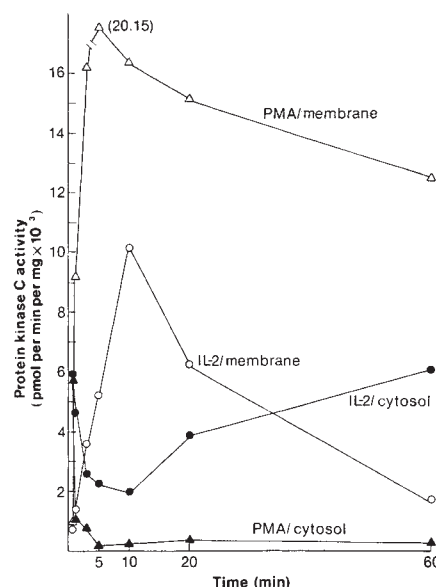


Fig. 1 Time course of association of PK-C with plasma membrane. IL-2-dependent CT6 cells were washed in serum-free RPMI-1640 medium and recultured in 1% fetal calf serum RPMI-1640 for 24 h. The cells were then washed twice and incubated at 37°C in serum-free RPMI-1640 for 2 h before stimulation. Cells (1×10^7) were resuspended in 1 ml in Eppendorf tubes and stimulated with either 100 ng PMA or 500 U of recombinant human IL-2 (Biogen). The cells were maintained in a shaking water bath at 37°C , removed at the indicated time intervals and washed three times in 1.0 ml serum-free RPMI-1640/1 mM sucrose. All liquids was absorbed from the cellular pellets and the cells were lysed in a 50- μl volume of distilled H_2O aspirated through a Hamilton syringe for 20 s. Immediately, the lysate was reconstituted with 1.0 ml ice-cold sample buffer (20 mM Tris pH 7.5, 0.33 M sucrose, 2 mM EDTA, 0.5 mM EGTA and 2 mM phenylmethylsulphonyl fluoride). Cytosol and particulate membrane fractions were prepared by centrifugation at 100,000 g for 60 min. Centrifuged membrane pellets were homogenized with a glass rod in 1% NP40 sample buffer, gently rotated for 30 min at 4°C , and centrifuged at 12,000 r.p.m. for 15 min. Approximately 500 μl was passed through a 1-ml DE52 column equilibrated with sample buffer minus sucrose. Columns were washed with 15 ml sample buffer, 0.5 ml of 0.08 M NaCl, and an additional 1.5 ml of 0.08 M NaCl eluate was collected; all the PK-C activity was found in this fraction¹¹. Aliquots (50 μl) were assayed for PK-C activity as described elsewhere^{11,12} with 1 mM CaCl_2 , 10 mM magnesium acetate, 100 μM [γ - ^{32}P]ATP (60 c.p.m. pmol^{-1}), 50 μg H1 histone, with or without 5 μg phosphatidylserine and 1 μg diolein. Data are expressed as PK-C-specific activity (pmol per min per mg) attributed to activity stimulated by phospholipid.

and toxins, appears to generate transmembrane control of cellular functions^{13–17}. Phosphatidylinositol (PI) turnover, specifically, has been shown to activate a novel protein kinase (PK-C) by the generation of a transient metabolite of PI degradation, diacylglycerol^{18,19}. The activation of PK-C seems to play a crucial part in the signal transduction elicited by extracellular messengers¹³. Although PK-C is normally found in a functionally inactive form in the cytosol of a variety of resting cell types, phorbol esters have been shown to stimulate a rapid protracted association of PK-C with plasma membrane, whereupon the lipid-rich environment plus Ca^{2+} serve as cofactors for the activation of enzymatic kinase activity^{11,12}. The process of phorbol ester activation of PK-C activity is believed to be independent of phosphatidylinositol hydrolysis and diacylglycerol formation. Phorbol esters apparently displace the physicochemical association between diacylglycerol and PK-C and, as a result, activate PK-C in the absence of phosphoinositides^{20,21}. Interleukin-2, however, probably acts through a phosphatidylinositol hydrolysis-dependent process, as indicated by

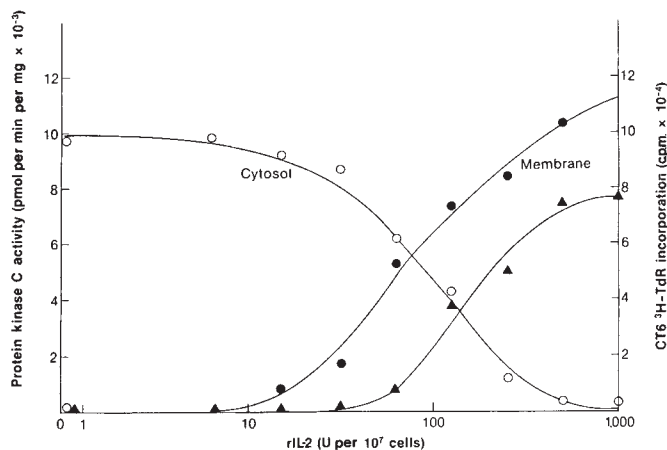


Fig. 2 Dose-response of IL-2-stimulated PK-C membrane association and cellular proliferation. CT6 cells were washed and prepared as described in Fig. 1 legend, then the indicated doses of recombinant IL-2 (rIL-2) were added and the cells were incubated for 10 min, followed by washing and lysis. Aliquots of the cell suspensions were taken and proliferation was measured in a standard IL-2 bioassay⁵. Proliferation data are expressed as the mean c.p.m. of triplicate cultures seeded with 1×10^4 cells per microculture (▲); PK-C activity in membrane (●) and cytosol (○) was determined as described in Fig. 1 legend and elsewhere¹¹.

the transient nature of the PK-C membrane association (Fig. 1). Preliminary experiments using isolated plasma membranes from IL-2-dependent cell lines have demonstrated that the principle metabolites formed on *in vitro* IL-2 stimulation of the isolated membranes were the PI metabolites phosphatidylinositol 4'-monophosphate and phosphatidylinositol 4', 5'-bisphosphate (data not shown). However, diacylglycerol, a neutral lipid, could not be recovered by the single-dimension TLC analysis employed. Even though diacylglycerol is generated by PI hydrolysis, it is rapidly converted to phosphatidic acid and arachidonic acid, which may therefore explain the membrane association/dissociation kinetics observed with IL-2. Phorbol esters, on the other hand, are not readily degraded enzymatically and therefore substitute for diacylglycerol for a more protracted period²².

Recent studies have shown that an initial event induced by growth stimulators is enhanced phosphorylation of specific membrane proteins^{23,24}. The products of several different viral transforming genes have also been demonstrated to be protein kinases^{25,26} and association with the plasma membrane seems to be required for *in vivo* tumour formation²⁷⁻²⁹. In addition, the Rous sarcoma virus transforming gene product, pp60^{v-src}, and the UR2 transforming protein of avian sarcoma virus have been shown to have lipid kinase activity which phosphorylates PI and diacylglycerol, suggesting a mechanism for transformation involving the initiation of PI turnover which, in turn, can activate several protein kinase activities including PK-C^{30,31}. Our studies suggest that IL-2 activates a protein phosphorylation system, PK-C, which is also associated with the biological activity of tumour promoters and certain oncogenes^{13-15,30,31}. The data are consistent with the notion that oncogenes may mimic or interact at different discrete events in phosphorylating systems that are normally stimulated by ligand-receptor interactions, and thereby initiate a cascading series of events associated with cellular proliferation. The finding that IL-2 initiates PK-C activation defines an event necessary for the growth of lymphoid cells, and allows the development of a model for the mechanism of action of as yet undefined oncogenic elements that may be involved in leukaemogenesis.

We thank Mr Harold Stull for technical assistance and Drs J. J. Oppenheim, R. B. Herberman and F. Ruscetti for helpful suggestions in the preparation of this manuscript.

Received 20 August 1984; accepted 23 January 1985.

- Ruscetti, F., Morgan, D. & Gallo, R. J. *Immun.* **119**, 131-138 (1977).
- Domzig, W., Stadler, B. M. & Herberman, R. B. *J. Immun.* **130**, 1970-1976 (1983).
- Smith, K. A. *Immun. Rev.* **51**, 337-357 (1983).
- Cantrell, D. A. & Smith, K. A. *Science* **224**, 1312-1316 (1984).
- Farrar, W. L., Johnson, H. M. & Farrar, J. J. *Immun.* **126**, 1120-1128 (1980).
- Robb, R. J., Murick, A. & Smith, K. A. *J. exp. Med.* **154**, 1445-1465 (1981).
- Cantrell, D. A. & Smith, K. A. *J. exp. Med.* **158**, 1895-1911 (1983).
- Oroz, C. G., Roopernian, D. C. & Bach, F. H. *J. Immun.* **130**, 1764-1771 (1983).
- Johnson, H. M., Torres, B. A., Archer, D. L. & Farrar, W. L. in *Interleukins, Lymphokines and Cytokines* (eds Oppenheim, J. & Cohen, S.) 51-62 (Academic, New York, 1983).
- Benjamin, W. R., Steeg, P. S. & Farrar, J. J. *Proc. natn. Acad. Sci. U.S.A.* **79**, 5379-5383 (1982).
- Kraft, A. S. & Anderson, W. B. *Nature* **301**, 621-623 (1983).
- Kraft, A. S., Anderson, W. B., Cooper, H. L. & Sando, J. J. *J. biol. Chem.* **257**, 13193-13196 (1982).
- Nishizuka, Y. *Nature* **308**, 693-698 (1984).
- Marx, J. L. *Science* **224**, 271-274 (1984).
- Michell, B. *Nature* **308**, 770 (1984).
- Michell, R. H. *Biochim. biophys. Acta* **415**, 81-147 (1975).
- Berridge, M. J. *Molec. cell. Endocr.* **24**, 115-140 (1978).
- Takai, Y., Kishimoto, A., Kikkawa, U., Mozi, T. & Nishizuka, Y. *Biochem. biophys. Res. Commun.* **91**, 1218-1224 (1979).
- Kishimoto, A., Takai, Y., Mozi, T., Kikkawa, U. & Nishizuka, Y. *J. biol. Chem.* **255**, 2273-2276 (1980).
- Castagna, M. et al. *J. biol. Chem.* **257**, 7847-7851 (1982).
- Yamanishi, J. et al. *Biochem. biophys. Res. Commun.* **112**, 778-786 (1983).
- Blumberg, P. M. *CRC Crit. Rev. Tox.* **8**, 153-197 (1980).
- Nilsen-Hamilton, M. & Hamilton, R. T. *Nature* **279**, 444-446 (1979).
- Cohen, S., Carpenter, G. & King, L. *J. biol. Chem.* **255**, 4834-4842 (1980).
- Ek, B., Westermark, B., Wasteson, A. & Heldin, C.-H. *Nature* **295**, 419-420 (1982).
- Erickson, R. L., Cullett, M. S., Erickson, E. & Purchio, A. F. *Proc. natn. Acad. Sci. U.S.A.* **76**, 6260-6264 (1979).
- Shih, T. Y., Papageorge, A. G., Stokes, P. E., Weaks, M. O. & Scolnick, E. M. *Nature* **287**, 686-691 (1980).
- Hynes, R. O. *Cell* **21**, 601-602 (1980).
- Kreuger, J. G., Garber, E. A., Goldberg, A. R. & Hanafuser, H. *Cell* **28**, 889-896 (1982).
- Sugimoto, Y., Whitman, M., Cantley, Z. C. & Erickson, R. L. *Proc. natn. Acad. Sci. U.S.A.* **81**, 2117-2121 (1984).
- Macara, I. G., Marinetti, G. V. & Balduzzi, P. C. *Proc. natn. Acad. Sci. U.S.A.* **81**, 2728-2732 (1984).

Altered cytosol/membrane enzyme redistribution on interleukin-3 activation of protein kinase C

William L. Farrar*, Thomas P. Thomas† & Wayne B. Anderson†

* Laboratory of Molecular Immunoregulation, National Cancer Institute-Frederick Cancer Research Facility, Frederick, Maryland 21701, USA

† Laboratory of Tumor Immunology and Biology, National Cancer Institute, Bethesda, Maryland 20205, USA

Interleukin-3 (IL-3) is a member of a family of growth and differentiation peptides, collectively referred to as colony-stimulating factors, which regulate haematopoiesis¹⁻³. IL-3 has been highly purified from medium conditioned by WEHI-3B cells, and recently the molecular cloning of complementary DNA for murine IL-3 has been reported^{4,5}. IL-3 seems to stimulate a wide range of colony-forming cells derived from murine bone marrow and has consequently been studied under a variety of names, including burst-promoting activity⁶, mast cell growth factor⁷, P-cell stimulating factor⁸ and multi-colony-stimulating factor^{3,9}. Here we present evidence that IL-3-receptor interaction stimulates the rapid and transient redistribution of protein kinase C (PK-C) from cytosol to plasma membrane in FDC-P1 cells. Phorbol myristate acetate (PMA) is shown to have a similar effect in these IL-3-dependent FDC-P1 cells. Our data suggest that IL-3 and phorbol esters share a common feature of transmembrane signalling crucial for growth and differentiation.

FDC-P1 cells were established originally as a cell line dependent for its sustained growth and viability on conditioned media derived from WEHI-3 cells¹⁰. Ihle and colleagues established that IL-3 is the principal factor within WEHI-3 conditioned media which is responsible for the growth promotion of FDC-P1 cells¹¹. In the present studies, FDC-P1 cells were washed free of support conditioned medium, rested for 2 h in serum-free media and stimulated with 100 ng PMA per 10^7 cells. At various times after PMA addition, the cells were washed, lysed and the

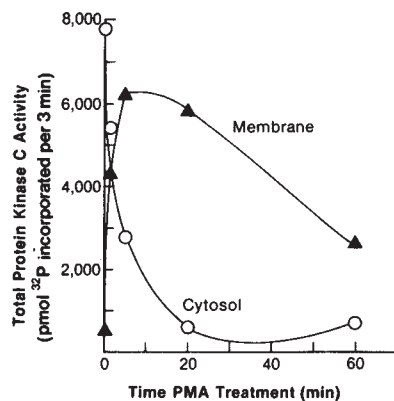


Fig. 1 Time course of the effect of PMA treatment of FDC-P1 cells on cytosolic (○) and particulate (▲) protein kinase C. FDC-P1 cells were grown in the presence of 10% FCS-RPMI 1640 and 20% WEHI-3 conditioned medium as described elsewhere¹¹. The cells were washed extensively, reconstituted with serum-free RPMI-1640 at a density of 1×10^7 per experimental point, then incubated for 2 h in a 37 °C water bath in Eppendorff tubes before stimulation with 100 ng PMA. After addition of PMA, at the indicated time intervals, cells were collected and washed rapidly three times with RPMI 1640, 1 M sucrose and pelleted in a micro-fuge. The cellular pellet was lysed within 20 s in 50 μ l of double-distilled water by passage through a narrow-gauge needle and immediately reconstituted with 1.0 ml ice-cold buffer A (20 mM Tris-HCl pH 7.5, 0.5 mM EGTA, 2 mM EDTA, 2 mM phenylmethylsulphonyl fluoride). Lysates were centrifuged at 100,000g and cytosol and membrane particulate fractions obtained. Particulate membrane fractions were solubilized with buffer A containing 1% NP40 detergent and both cytosolic and solubilized membrane fractions were fractionated by DEAE-cellulose chromatography as described in detail elsewhere¹⁴. Fractions eluting with 0.08 M NaCl contained all the detectable PK-C activity¹⁴. Aliquots (50 μ l) of DEAE-purified material were assayed for cytosolic and particulate Ca^{2+} , phospholipid-dependent PK-C activity in the presence of 10 mM Mg acetate, 0.75 mM CaCl_2 , 100 μ M [γ -³²P]ATP (60 c.p.m. pmol⁻¹) and 25 μ g of histone H1, with or without 24 μ g phosphatidylserine and 1.6 μ g diolein¹⁴. Protein kinase activity was determined by subtracting the amount of ³²P incorporation into histone noted in the absence of added phospholipids, from the amount of ³²P incorporation noted in the presence of phospholipids. Total protein kinase C activity is expressed as the pmol ³²P incorporated per 3 min per total volume of each cytosolic and membrane preparation.

Ca^{2+} , phospholipid-dependent PK-C activity which was measured in the cytosol and detergent-solubilized membrane fractions was isolated.

Figure 1 shows a time-kinetic comparison of the total PK-C activity in cytosol and membrane fractions of FDC-P1 cells treated with PMA. Within 1–2 min of PMA addition, cytosolic PK-C activity had begun to fall rapidly; it had fallen 10-fold within 20 min of incubation in the presence of PMA and remained depressed for the remainder of the time course. Concomitant with the decrease in cytosolic PK-C activity, there was an equally rapid (1–2 min) increase in membrane-associated PK-C resulting in a 10-fold increase in total enzyme activity by 10 min post-treatment. Examination of the total cellular enzyme activity (membrane + cytosol) revealed no changes in total PK-C activity with PMA stimulation (data not shown). Rather, the data suggested that PMA mediates a redistribution of PK-C activity from cytosol to membrane. These findings are consistent with previous studies demonstrating similar PK-C translocations from cytosol to plasma membrane in phorbol ester-treated murine thymoma EL4 cells, interleukin-2 (IL-2)-dependent CT6 cells and parietal yolk sac cells (PYS)^{12–15}.

FDC-P1 cells were washed free of support WEHI-3 conditioned medium, incubated for 2 h at 37 °C in serum-free RPMI 1640, stimulated with 100 U of purified IL-3 (U = reciprocal dilution at half-maximal proliferation)⁴ and the time course of

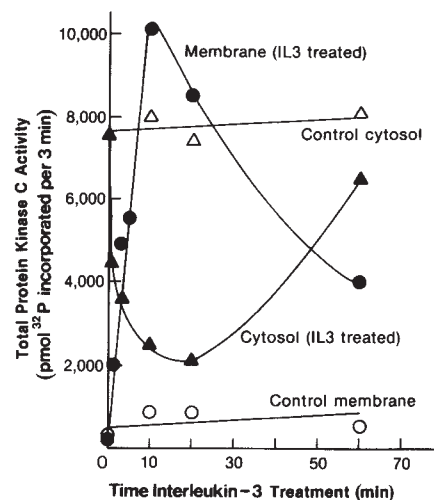


Fig. 2 Time course of the effect of IL-3 treatment of FDC-P1 cells on cytosolic and particulate protein kinase C. Washed FDC-P1 cells were prepared as for Fig. 1. Following preincubation in serum-free medium, 10^7 cells were stimulated with 100 U of homogeneous IL-3 prepared from WEHI-3 conditioned media⁴. Other cells were incubated in the absence of IL-3 to serve as the control cell population. At the indicated time intervals, the cells were washed, lysed and centrifuged at 100,000g to prepare cytosolic (△, ▲) and membrane (○, ●) fractions from control (closed symbols) and IL-3-treated (open symbols) cells, as described in Fig. 1 legend. Cytosol and NP40 detergent-solubilized particulate preparations were applied to and eluted from DE52-cellulose columns and protein kinase C activity was determined in the presence and absence of phospholipids as described for Fig. 1.

cytosol- and membrane-associated PK-C activity was evaluated (Fig. 2). Unstimulated FDC-P1 cells contained ~7,500 U PK-C cytosolic activity (1 U = 1 pmol ³²P incorporated in 3 min). Treatment with IL-3 resulted in a precipitous decrease in cytosolic PK-C activity within 1–3 min (from 7,500 to 3,500 U), with a greater than threefold decrease within 20 min post-IL-3 treatment. This IL-3-stimulated decline of cytosolic PK-C activity was transient, however, and was followed by a return to near control levels by 60 min post-stimulation. Control cytosolic PK-C activity (no IL-3 added) did not change significantly ($\pm 15\%$) during FDC-P1 cell incubation, and total control membrane-associated detergent-solubilized PK-C activity was considerably lower (100–200 U) than control PK-C cytosolic activity (7,500 U).

Concomitant with the rapid loss of cytosol PK-C activity was an equally marked increase in membrane-associated activity, resulting in a >10-fold increase in membrane-associated activity within 10–20 min post-IL-3 treatment. Control (no IL-3 added) membrane PK-C activity did not change significantly during cell incubation. The return (increase) of cytosolic PK-C activity 20–60 min after IL-3 addition was associated with a corresponding decrease of membrane-associated activity. Total cellular (membrane + cytosol) PK-C activity did not change significantly during the exposure to IL-3, suggesting that the reciprocal kinetics of cytosolic and membrane-associated PK-C activity results from IL-3-mediated PK-C enzyme translocation between soluble and particulate fractions.

Figure 3 depicts the dose-response curve noted for IL-3-stimulated DNA synthesis relative to the membrane-association kinetics of PK-C activity in FDC-P1 cells. Incubation of FDC-P1 cells for 15 min with increasing concentrations of IL-3 (to 500 U) caused a progressive, IL-3 dose-dependent decrease in cytosolic PK-C activity, while particulate PK-C activity increased. The increase in detergent-solubilized PK-C activity in response to IL-3 was proportional to the dosage of IL-3 used to treat the FDC-P1 cells. The proliferative dose-response of FDC-P1 cells to IL-3 paralleled the membrane-association kinetics of PK-C,

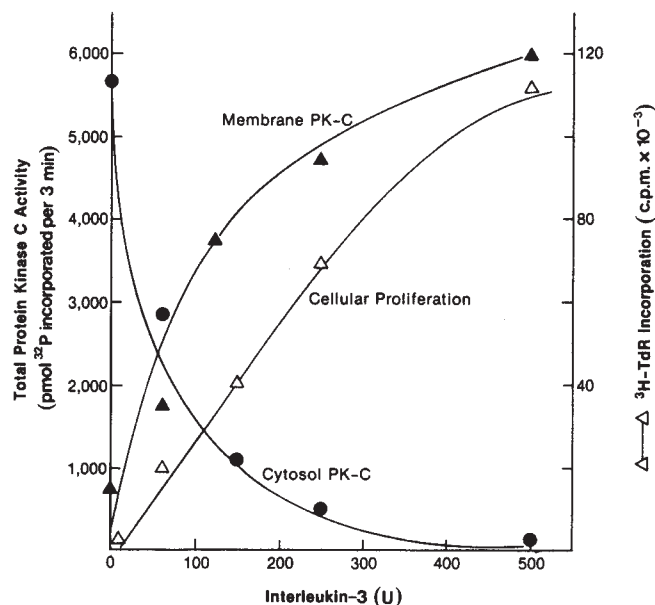


Fig. 3 Effect of treatment of FDC-P1 cells with increasing concentrations of IL-3 on the subcellular redistribution of protein kinase C activity and on DNA synthesis. To determine the dose response of IL-3 on PK-C activity, FDC-P1 cells were incubated with the indicated dosages of purified IL-3 for 15 min. The cells were washed and cytosol (●) and particulate (▲) membrane fractions prepared as for Fig. 1. Cytosolic and detergent-solubilized membrane preparations were separated by DEAE-cellulose chromatography to determine the total PK-C activity as described in Fig. 1 legend. Cellular proliferation (△) was determined by measuring the incorporation of ³H-thymidine (TdR) as described elsewhere¹¹. Cellular proliferation is expressed as the mean c.p.m. of triplicate cultures of 3×10^6 cells per culture.

Received 20 October 1984; accepted 15 January 1985.

1. Schrader, J. W. *CRC crit. Rev. Immun.* **4**, 197-277 (1983).
2. Howard, M., Burgess, A. W., McPhee, D. & Metcalf, D. *Cell* **18**, 993-999 (1980).
3. Nicola, N. A. & Vadas, M. *Immun. Today* **5**, 76-80 (1984).
4. Ihle, J. N. *et al. J. Immun.* **131**, 282-287 (1983).
5. Fung, M. C. *et al. Nature* **307**, 233-236 (1984).
6. Iscove, N. N., Roitsch, C. A., Williams, N. & Guilbert, L. J. *J. cell. Physiol. Suppl.* **1**, 65-78 (1982).
7. Nabel, G., Galli, S. J., Dvorak, A. M., Dvorack, H. F. & Cantor, H. *Nature* **291**, 332-334 (1981).
8. Schrader, J. W., Lewis, S. J., Clark-Lewis, I. & Culvenor, J. G. *Proc. natn. Acad. Sci. U.S.A.* **78**, 323-327 (1981).
9. Bazill, C. W., Haynes, M., Garland, J. & Dexter, T. M. *Biochem. J.* **210**, 747-759 (1983).
10. Dexter, T. M., Garland, J., Scott, D., Scolnick, E. & Metcalf, D. *J. exp. Med.* **152**, 1036-1046 (1980).
11. Ihle, J. N. *et al. J. Immun.* **129**, 1377-1383 (1982).
12. Farrar, W. L. & Anderson, W. B. *Nature* **315**, 233-235 (1985).
13. Kraft, A. S. & Anderson, W. B. *Nature* **301**, 621-623 (1983).
14. Kraft, A. S., Anderson, W. B., Cooper, H. L. & Sando, J. J. *J. biol. Chem.* **257**, 13192-13196 (1982).
15. Nishizuka, Y. *Nature* **308**, 693-698 (1984).
16. Anderson, W. B. & Saloman, D. *Phospholipids and Cellular Regulation* (ed. Kuo, J. F.) (CRC Press, Boca Raton, Florida, in the press).
17. Sugimoto, Y., Whitman, M., Cantley, Z. C. & Erickson, R. L. *Proc. natn. Acad. Sci. U.S.A.* **81**, 2116-2121 (1984).
18. Macara, I. G., Marinetti, G. V. & Balduzzi, P. C. *Proc. natn. Acad. Sci. U.S.A.* **81**, 2728-2732 (1984).
19. Berridge, M. J. & Irvine, R. F. *Nature* **312**, 315-321 (1984).
20. Waterfield, M. D. *et al. Nature* **304**, 35-39 (1983).
21. Downward, J. *et al. Nature* **307**, 521-527 (1984).

Selective inhibition of the anchorage-independent growth of *myc*-transfected fibroblasts by retinoic acid

Anita B. Roberts, Nanette S. Roche & Michael B. Sporn

Laboratory of Chemoprevention, National Cancer Institute, Bethesda, Maryland 20205, USA

and the IL-3 ED₅₀ for both the increase in membrane-associated PK-C activity and FDC-P1 DNA synthesis was within the same dose range (200-250 U). These results suggest a quantitative relationship between PK-C membrane association and the proliferative response of FDC-P1 cells to IL-3.

The data presented here demonstrate that a haematopoietic growth factor, IL-3, stimulates a rapid and transient increase in the redistribution of PK-C activity from cytosol to the membrane (Fig. 2). IL-3 probably acts through a phosphoinositide hydrolysis-dependent mechanism involving the generation of diacylglycerol, as indicated by the transient nature of the membrane association. Phorbol esters, however, are not readily degraded enzymatically and may, therefore, become associated with the enzyme in place of the apparent physiological regulator for a protracted period, which would allow a more prolonged association of PK-C with the membrane and override other regulatory capabilities of the cell (such as the rapid metabolism of diacylglycerol) (Fig. 1)^{16,17}.

In related studies, IL-2, a lymphocytotropic growth factor, also stimulates transient PK-C subcellular redistribution¹². The similarity of the IL-2-induced PK-C activation process and IL-3 suggests that growth or progression peptides share common features of transmembrane biochemical signals involved in growth promotion. Interestingly the viral transforming gene products *src* and *ros* have recently been demonstrated to possess lipid kinase activity^{18,19}. This would presumably result both in the activation (and membrane association) of PK-C in response to increased diacylglycerol levels and to increased intracellular Ca²⁺ levels in response to the polyphosphoinositides^{16,20}. Taken together, the data are consistent with the hypothesis that oncogenic elements mimic certain ligand-receptor interactions, thereby initiating a series of transmembrane phosphorylation events which are normally triggered by extracellular growth peptides¹⁹⁻²¹.

Fischer rat 3T3 (FR3T3) fibroblasts transfected with a cellular *myc* gene¹ can be induced to grow and form colonies in soft agar by treatment either with epidermal growth factor (EGF) alone or with the combination of platelet-derived growth factor (PDGF) and type-β transforming growth factor (TGF-β)². We now show that induction of anchorage-independent growth by each of these sets of growth factors involves different cellular pathways which can be distinguished by their sensitivity to retinoic acid. Colony formation induced by the combined action of PDGF and TGF-β is 100-fold more sensitive to inhibition by retinoic acid than is colony formation induced by treatment of the *myc*-transfected cells with EGF. Moreover, retinoic acid (10⁻⁸ M) is inhibitory for colony growth whenever TGF-β is present, regardless of whether the effects of TGF-β are stimulatory, as occurs in the presence of PDGF, or inhibitory, as found in the presence of EGF².

The cellular mechanisms of induction of anchorage-independent growth by peptide growth factors such as PDGF³⁻⁵, TGF-β⁶⁻⁸ and the TGF-α family⁹⁻¹¹ (which includes EGF) are poorly understood. These three distinct families of growth factors¹²⁻¹⁵ share the ability to induce phenotypic transformation of non-neoplastic fibroblasts, as measured by growth in soft agar¹⁶⁻¹⁸. The increased secretion of TGF-α^{19,20}, PDGF²¹ and TGF-β²² which accompanies virus transformation of cells provides further evidence for the role of these peptides in expression of the transformed phenotype. It has recently become apparent that the product of the *myc* gene, acting in the nucleus, is involved in the pathway by which a mitogenic stimulus is generated by a growth factor acting on its receptor²³⁻²⁸. For example, treatment of quiescent fibroblasts with PDGF, which induces a state competent for replication²⁹, results in transient expression of the *myc* gene³⁰. Consistent with this observation, BALB/c 3T3 cells transfected with *myc* partially lose their requirement for PDGF³¹. Thus, whereas control cells require both PDGF and EGF for growth in monolayer culture in plasma²⁹, *myc*-transfected cells can grow in plasma in the presence of EGF alone³¹.

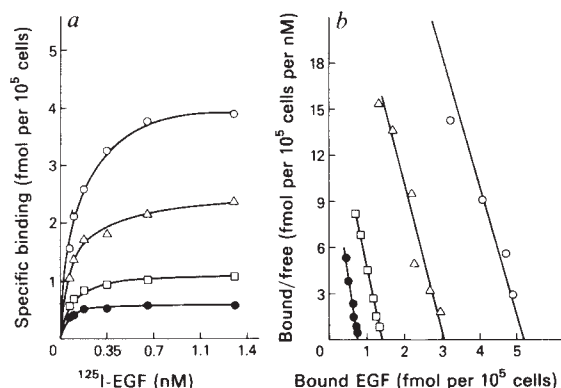


Fig. 1 Retinoic acid increases the number of cell-surface EGF receptors on Myc-1 cells. *a*, Binding of ¹²⁵I-labelled EGF (150 μCi μg⁻¹) to Myc-1 cells grown either in the absence of added retinoic acid (●) or in the presence of 10⁻⁸ M (□), 10⁻⁷ M (△) or 10⁻⁶ M (○) retinoic acid. *b*, Scatchard plot of the data in *a*.

Methods. The Myc-1 cell line was derived from FR3T3 fibroblasts by co-transfection of a neomycin-selectable marker gene and a construct in which exons 2 and 3 of a cellular *myc* gene derived from a mouse plasmacytoma were put under control of the SV40 early promoter (plasmid pSVc-myc-1)¹. Cells were seeded in 1 ml of Dulbecco's modified Eagle's medium containing 5% calf serum at a density of 1 × 10⁴ cells per 16-mm multiwell dish. Retinoic acid (in 20 μl of a 2% solution of dimethyl sulphoxide in medium) was added 4 h after seeding and the incubation was continued for 60 h before the binding assay³⁷. Binding of ¹²⁵I-labelled EGF was measured at room temperature in the absence of retinoic acid. Nonspecific binding, measured in the presence of unlabelled EGF at a concentration of 1 μg ml⁻¹, was subtracted from all values.

Results are the average of triplicate determinations.

Transfected NIH 3T3 or Rat-2 cells expressing an activated *myc* gene are tumorigenic and have an increased, though low (1%), cloning efficiency in soft agar compared with control cells³². We have shown that FR3T3 cells transfected with exons 2 and 3 of a cellular *myc* gene under control of the simian virus 40 (SV40) early promoter¹ (Myc-1 cells) do not grow in soft agar, but can be induced to form colonies by the addition of growth factors²; control cells transfected with the neomycin marker alone cannot form colonies. Myc-1 cells require only EGF to form colonies², and PDGF has little additional effect on cell growth in the presence of optimal concentrations of EGF. In contrast, PDGF is essential for anchorage-independent growth of Myc-1 cells in the presence of TGF-β³. This suggests that one function of PDGF may be to induce expression of other genes³³ in addition to *myc* which are required in the signalling pathways of TGF-β.

To probe for possible mechanistic differences in the action of EGF and TGF-β on anchorage-independent growth of Myc-1 cells, we investigated the effects of retinoic acid on the growth factor-dependent colony formation of the cells. Retinoic acid is known to inhibit colony growth of certain tumour cells and *in vitro* transformation of fibroblasts (reviewed in ref. 34). Previous studies suggest that retinoic acid³⁵⁻³⁷ and TGF-β³⁸ may share common mechanistic elements, since they both increase the number of EGF receptors of normal rat kidney (NRK) cells. In the present study, retinoic acid treatment (60 h) of Myc-1 cells similarly resulted in a dose-dependent increase in the number of cellular EGF receptors, 10⁻⁸ M, 10⁻⁷ M and 10⁻⁶ M retinoic acid producing a respective two-, four- and seven-fold increase in the number of EGF receptors (Fig. 1*a,b*), with no apparent effect on receptor affinity. However, in contrast to the stimulatory effects of this increased number of EGF receptors on soft agar growth of NRK cells³⁵⁻³⁷, treatment of Myc-1 cells with retinoic acid (10⁻⁸ M) had little effect on the dose-dependent induction of anchorage-independent growth by EGF (Fig. 2*a*). Concentrations of retinoic acid >10⁻⁸ M inhibited EGF-induced colony formation; 10⁻⁷ and 10⁻⁶ M retinoic acid produced 50 and 85% inhibition, respectively (Fig. 2*c*). Retinoic acid treatment of control FR3T3 cells transfected with the

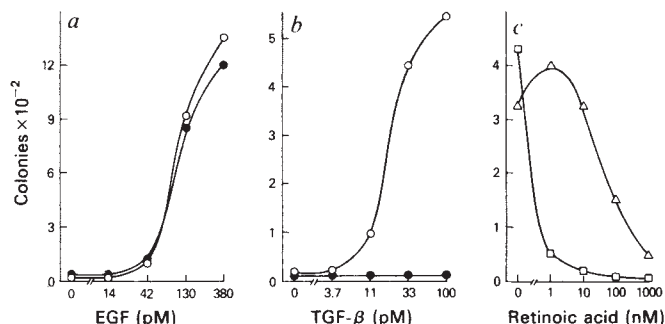


Fig. 2 Selective inhibition by retinoic acid of the anchorage-independent growth of Myc-1 fibroblasts in the presence of PDGF and TGF-β. *a*, Colony growth assayed in the presence of EGF alone. *b*, Colony growth assayed in the presence of 150 pM PDGF and the concentration of TGF-β indicated. *c*, Colony growth assayed in the presence of 42 pM EGF alone (△) or the combination of 150 pM PDGF and 100 pM TGF-β (□) and varying concentrations of retinoic acid. Myc-1 cells were assayed for the formation of colonies of >62 μm diameter after 7 days' incubation in soft agar in Dulbecco's modified Eagle's medium, 10% calf serum as described previously³⁷. Purified murine EGF, human PDGF or human platelet TGF-β⁷ were added at the time of the assay² in the presence (●) or absence (○) of 10⁻⁸ M retinoic acid.

neomycin marker resulted in an increase in EGF binding similar to that observed in Myc-1 cells; however, the increased binding did not alter the inability of these cells to grow in soft agar either in the presence or absence of added growth factors (not shown). This suggests that these effects of retinoic acid on increasing the number of EGF receptors of transfected FR3T3 cell lines are unrelated to its effects on EGF-dependent colony growth of the cells in soft agar.

Remarkably, colony formation stimulated by treatment of Myc-1 cells with PDGF and TGF-β is ~100-fold more sensitive to inhibition by retinoic acid than is the EGF-dependent colony formation (Fig. 2*c*). Thus retinoic acid (10⁻⁸ M) is a potent inhibitor of colony formation stimulated by treatment of the cells with PDGF and TGF-β (Fig. 2*b*). The inhibition could not be overcome by increasing the TGF-β concentration as high as 100 pM (Fig. 2*b*), and concentrations of retinoic acid as low as 10⁻⁹ M still inhibited colony formation by >90% (Fig. 2*c*). Retinoic acid had little or no effect on either the number or affinity of cellular TGF-β receptors (not shown).

To study separately the effects of retinoic acid on the action of either PDGF or TGF-β on Myc-1 cells, we examined the effects of 10⁻⁸ M retinoic acid on colony growth in the presence of EGF with additions of either PDGF or TGF-β (Fig. 3*a, b*). PDGF slightly potentiated the action of suboptimal levels of EGF on colony formation (Fig. 3*a*), whereas we had shown previously that TGF-β partially inhibits the effect of EGF on growth in soft agar (ref. 2 and Fig. 3*b*). Retinoic acid had either no effect or slightly stimulated the colony growth induced by PDGF in the presence of 42 pM EGF (Fig. 3*a*). However, retinoic acid synergized with TGF-β to abolish the colony formation induced by treatment of Myc-1 cells with 420 pM EGF (Fig. 3*b*). Thus, in the absence of retinoic acid, TGF-β (10–100 pM) inhibits EGF-dependent colony formation to a maximum of 60% (ref. 2 and Fig. 3*b*), but when Myc-1 cells are treated with 10⁻⁸ M retinoic acid, which by itself has no effect on EGF-dependent colony formation (Fig. 2*a*), concentrations of TGF-β >~10 pM result in nearly total inhibition of the colony-forming response (Fig. 3*b*).

The discriminating action of low concentrations of retinoic acid on growth factor-dependent colony formation by Myc-1 cells was observed only in conditions of anchorage-independent growth. Growth of the cells in monolayer culture was consistently inhibited by TGF-β and either unaffected or stimulated by retinoic acid (10⁻⁷ M), regardless of the particular combination of growth factors added (Table 1). Thus, the selective action of retinoic acid cannot be related mechanistically to the growth of cells *per se*, but rather, specifically to its effects on processes

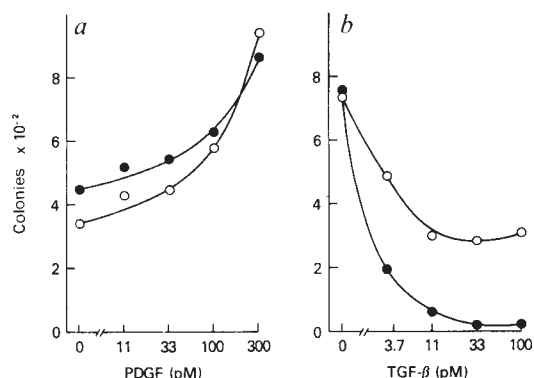


Fig. 3 Inhibition by retinoic acid is dependent on the presence of TGF- β . *a*, Myc-1 cells assayed in the presence of 42 pM EGF and increasing concentrations of PDGF. *b*, Colony growth assayed in the presence of 420 pM EGF and increasing concentrations of TGF- β . Myc-1 cells were assayed for colony formation in soft agar in the presence (●) or absence (○) of 10^{-8} M retinoic acid as described in Fig. 2 legend.

Table 1 Effects of retinoic acid on growth of Myc-1 cells in monolayer culture

Additions	No. of cells per dish ($\times 10^{-4}$)	
	-Retinoic acid	+Retinoic acid (10^{-7} M)
None	6.6	6.5
EGF (800 pM)	10	13
PDGF (200 pM)	15	19
TGF- β (4 pM)	1.2	1.6
EGF+TGF- β	2.2	4.8
PDGF+TGF- β	7.3	13

Cells were seeded into 16-mm multiwell dishes at a density of 1×10^4 cells per well in 1 ml of Dulbecco's modified Eagle's medium containing 2% calf serum. Retinoic acid, in 20 μ l 2% dimethyl sulphoxide in medium, and growth factors, in 10–20 μ l 4 mM HCl, were added 16 h after seeding. Cell counts were determined in triplicate in a Coulter counter 4 days after seeding.

correlated with transformation of cells, namely anchorage independence³⁹.

Although PDGF, EGF and TGF- α have a consistent stimulatory effect on the growth of cells in both anchorage-dependent and independent conditions, the actions of TGF- β on cells are known to be bifunctional^{2,40}. For example, we have shown here that TGF- β stimulates the anchorage-independent growth of Myc-1 cells in the presence of PDGF (Fig. 2*b*), but inhibits both the anchorage-dependent growth of the cells in the presence of PDGF or EGF (Table 1) and colony formation in the presence of EGF (Fig. 3*b*). Recently, TGF- β has been shown to be similar, or possibly identical, to a growth inhibitor isolated from African green monkey kidney cells⁴¹. The results presented here—that retinoic acid can selectively modulate the effects of TGF- β on *myc*-transfected cells—emphasize the distinctive mode of action of this growth factor and suggest that mitogenic effects of TGF- β on growth of cells in soft agar use different intracellular signalling pathways from those involved in transduction of mitogenic stimuli from either PDGF or EGF and their receptors. Most significantly, these results suggest that retinoic acid can be used to discriminate between different mechanisms of cellular transformation and between different pathways of growth factor action on cells.

We thank Drs David F. Stern and Robert Weinberg for providing the Myc-1 cell line, Dr Richard Assoian for helpful discussions, Dr Gary Grotendorst for providing PDGF, and Mr Joseph M. Smith and Dr Lalage M. Wakefield for assistance with the binding assays.

Received 11 December 1984; accepted 6 March 1985.

- Land, H., Parada, L. F. & Weinberg, R. A. *Nature* **304**, 596–602 (1983).
- Roberts, A. B. *et al. Proc. natn. Acad. Sci. U.S.A.* **82**, 119–123 (1985).

- Heldin, C.-H., Westermark, B. & Wasteson, A. *Biochem. J.* **193**, 907–913 (1981).
- Raines, E. W. & Ross, R. *J. biol. Chem.* **257**, 5154–5160 (1982).
- Waterfield, M. D. *et al. Nature* **304**, 35–39 (1983).
- Frolik, C. A., Dart, L. L., Meyers, C. A., Smith, D. M. & Sporn, M. B. *Proc. natn. Acad. Sci. U.S.A.* **80**, 3676–3680 (1983).
- Assoian, R. K., Komoriya, A., Meyers, C. A., Miller, D. M. & Sporn, M. B. *J. biol. Chem.* **258**, 7155–7160 (1983).
- Roberts, A. B. *et al. Biochemistry* **22**, 5692–5698 (1983).
- Marquardt, H. *et al. Proc. natn. Acad. Sci. U.S.A.* **80**, 4684–4688 (1983).
- Derynck, R., Roberts, A. B., Winkler, M. E., Chen, E. Y. & Goeddel, D. V. *Cell* **38**, 287–297 (1984).
- Roberts, A. B., Frolik, C. A., Anzano, M. A. & Sporn, M. B. *Fedn Proc.* **42**, 2621–2625 (1983).
- Heldin, C. H., Ek, B. & Ronnstrand, L. *J. biol. Chem.* **258**, 10054–10061 (1983).
- Massagué, J. *J. biol. Chem.* **258**, 13614–13620 (1983).
- Frolik, C. A., Wakefield, L. M., Smith, D. M. & Sporn, M. B. *J. biol. Chem.* **259**, 10995–11000 (1984).
- Tucker, R. F., Brannum, E. L., Shipley, G. D., Ryan, R. J. & Moses, H. L. *Proc. natn. Acad. Sci. U.S.A.* **81**, 6757–6761 (1984).
- Assoian, R. K., Grotendorst, G. R., Miller, D. M. & Sporn, M. B. *Nature* **309**, 804–806 (1984).
- Moses, H. L., Childs, C. B., Jaroslava, H., Shipley, G. D. & Tucker, R. F. in *Control of Cell Growth and Proliferation* (ed. Venziale, C. M.) 147–167 (Van Nostrand-Reinhold, New York, 1984).
- Kaplan, P. L. & Ozanne, B. *Cell* **33**, 931–938 (1983).
- De Larco, J. E., Preston, Y. A. & Todaro, G. J. *J. cell. Physiol.* **109**, 143–152 (1981).
- Kaplan, P. L., Anderson, M. & Ozanne, B. *Proc. natn. Acad. Sci. U.S.A.* **79**, 485–489 (1982).
- Bowen-Pope, D. F., Vogel, A. & Ross, R. *Proc. natn. Acad. Sci. U.S.A.* **81**, 2396–2400 (1984).
- Anzano, M. A. *et al. Molec. cell. Biol.* **5**, 242–247 (1985).
- Hann, S. R., Abrams, H. D., Rohrschneider, L. R. & Eisenman, R. N. *Cell* **34**, 789–798 (1983).
- Persson, H. & Leder, P. *Science* **225**, 718–721 (1984).
- Goyette, M., Petropoulos, C. J., Shank, P. R. & Fausto, N. *Molec. cell. Biol.* **4**, 1493–1498 (1984).
- Campisi, J., Gray, H. E., Pardee, A. B., Dean, M. & Sonenshein, G. E. *Cell* **36**, 241–247 (1984).
- Gonda, T. J. & Metcalf, D. *Nature* **310**, 249–251 (1984).
- Westin, E. H. *et al. Proc. natn. Acad. Sci. U.S.A.* **79**, 2490–2494 (1982).
- Stiles, C. D. *et al. Proc. natn. Acad. Sci. U.S.A.* **76**, 1279–1283 (1979).
- Kelly, K., Cochran, B. H., Stiles, C. D. & Leder, P. *Cell* **35**, 603–610 (1983).
- Armelin, H. A. *et al. Nature* **310**, 655–660 (1984).
- Keath, E. J., Caimi, P. G. & Cole, M. D. *Cell* **39**, 339–348 (1984).
- Cochran, B. H., Reffel, A. C. & Stiles, C. D. *Cell* **33**, 939–947 (1983).
- Roberts, A. B. & Sporn, M. B. in *The Retinoids* Vol. 2 (eds Sporn, M. B., Roberts, A. B. & Goodman, D. W. S.) 209–286 (Academic, New York, 1984).
- Jetten, A. M. *Cancer Res.* **43**, 68–72 (1983).
- Jetten, A. M. & Goldfarb, R. H. *Cancer Res.* **43**, 2094–2099 (1983).
- Roberts, A. B., Anzano, M. A., Lamb, L. C., Smith, J. M. & Sporn, M. B. *Cancer Res.* **44**, 1635–1641 (1984).
- Assoian, R. K., Frolik, C. A., Roberts, A. B., Miller, D. M. & Sporn, M. B. *Cell* **36**, 35–41 (1984).
- Kahn, P. & Shin, S. *J. Cell Biol.* **82**, 1–16 (1979).
- Moses, H. L. *et al. Cold Spring Harb. Cancer Cells* (in the press).
- Tucker, R. F., Shipley, G. D., Moses, H. L. & Holley, R. W. *Science* **226**, 705–707 (1984).

Association of phosphatidylinositol kinase activity with polyoma middle-T competent for transformation

Malcolm Whitman*, David R. Kaplan†, Brian Schaffhausen†, Lewis Cantley* & Thomas M. Roberts†

* Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts 02138, USA
 † Laboratory of Neoplastic Disease Mechanisms and Department of Pathology, Dana Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts 02115, USA
 ‡ Department of Biochemistry and Pharmacology, Tufts University Medical School, Boston, Massachusetts 02111, USA

Polyoma middle-T antigen is required for viral transformation of cultured cells and for tumorigenesis in animals. Like many other transforming gene products, middle-T is bound to the membrane and has an associated tyrosine kinase activity *in vitro*^{1,2}. This activity seems to result from the interaction of middle-T with pp60^{c-src}, the cellular homologue of the transforming gene product of the Rous sarcoma virus, pp60^{v-src} (refs 3–5). Both pp60^{v-src} (ref. 6) and another retrovirus transforming gene product, pp68^{v-ros} (ref. 7) were shown recently to have an associated phosphatidylinositol (PI) kinase activity *in vitro* and to increase PI turnover *in vivo*. These results suggest that viral transformation may be directly connected to a complex network of second messengers generated from PI turnover^{8–18}. Here, we assayed for PI kinase activity in immunoprecipitates made with middle-T- or pp60^{c-src}-specific antisera of cells infected with polyoma virus. A PI kinase activity was detected in those immunoprecipitates which contained middle-T. Studies of mutants of middle-T defective in transformation indicate a close correlation between PI kinase activity and transformation.

Immunoprecipitates of polyoma middle-T from infected 3T3 cells catalyse phosphorylation of PI. Figure 1a shows the relative PI phosphorylating ability of immunoprecipitates obtained on treatment of mock-infected or polyoma-infected cells with polyoma-specific serum or preimmune serum. Immunoprecipitates made from polyoma-infected 3T3 cells with anti-polyoma serum (Fig. 1a, lane 4) produced 20-fold more phosphatidylinositol diphosphate (DPI) after a 20-min incubation than did immunoprecipitates made from mock-infected cells (lane 3). No increase in PI phosphorylation above background levels was seen when immunoprecipitates were made with preimmune serum (Fig. 1a, lanes 1, 2). Quantitation of the PI phosphorylation in 10 separate experiments gave 10–50-fold enhancement for middle-T immunoprecipitates over controls. Similar results were observed when polyoma-infected 3T6 cells, 293 cells infected with an adenovirus expressing the middle-T gene, and a polyoma middle-T transformed cell line (py14-2)¹⁹ were compared with respective parent line or mock-infected controls (data not presented).

If the observed PI kinase activity is linked to the transformation activity of middle-T, virus mutations reducing transforming activity should reduce or eliminate the observed PI kinase activity of infected cell immunoprecipitates. Polyoma middle-T mutants defective in transformation fall into two categories: mutations that abolish transformation totally, like py1387T and NG59, and mutations that reduce but do not eliminate transforming activity, such as py1178T. py1387T contains a nonsense mutation which eliminates the last 37 amino acids at the C-terminus of middle-T, rendering it completely defective for transformation, membrane association and tyrosine kinase activity. Small-T is unaltered and large-T contains a single amino-acid substitution but is unaffected in its function²⁰. Figure 1b shows the effect of this mutation on viral stimulation of immunoprecipitated PI kinase activity. Immunoprecipitates from cells infected with py1387T (Fig. 1b, lane 2) showed no increase in PI kinase activity over mock-infected control (lane a). Parallel infections and immunoprecipitations from ³⁵S-methionine-labelled cells indicated that py1387T-infected cells contain similar levels of large-T, middle-T and small-T to those seen in wild-type polyoma-infected cells (not shown). As judged by ³H-thymidine uptake, py1387T and wild-type polyoma infection caused similar stimulation of DNA synthesis (data not shown). These results suggest that the PI kinase activity associated with polyoma immunoprecipitates may be linked to the transforming function of the virus and is not a consequence of cell stimulation by viral infection.

Experiments with the transformation-defective mutant NG59 indicate that the PI kinase activity associated with wild-type middle-T immunoprecipitates is not a consequence of membrane contaminants nonspecifically co-precipitated with membrane-bound middle-T. Like py1387T, NG59 is totally transformation defective, but unlike py1387T, the middle-T protein of NG59 associates with the membrane²¹. The mutation is a replacement of an aspartic acid residue at position 179 by isoleucine-asparagine. No PI kinase activity above background was detected using immunoprecipitates from cells infected with this virus (Table 1), even though ³⁵S-methionine labelling indicated that normal levels of mutant middle-T were present in the immunoprecipitates (not shown). Therefore, the PI kinase activity associated with middle-T is not merely a consequence of middle-T membrane association.

Unlike middle-T antigens produced by NG59 and py1387T mutants, which are totally inactive in transformation and in the standard middle-T tyrosine kinase assay *in vitro*, the middle-T product of the py1178T mutant virus (in which a Phe is substituted for Tyr 315) retains some associated tyrosine kinase activity and is partially defective in transformation^{22,23}. If PI kinase activity plays an important part in transformation, then immunoprecipitates of middle-T prepared from cells infected with this virus should be less active in PI phosphorylation than wild-type immunoprecipitates; this was observed (Fig. 2a). Although py1178T immunoprecipitates contained some PI

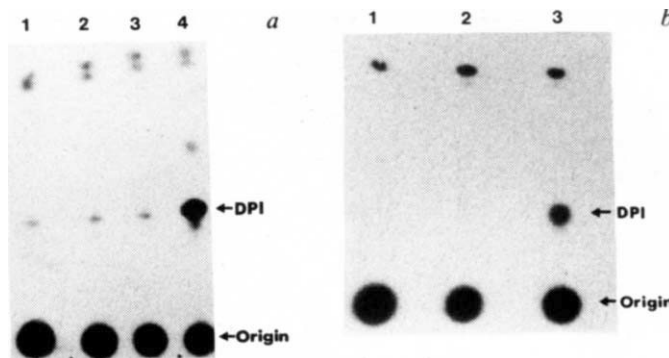


Fig. 1 Phosphorylation of phosphatidylinositol by polyoma immunoprecipitates. *a*, Autoradiograph of a TLC separation of the reaction products from PI kinase assays performed on immunoprecipitates. Lane 1, mock-infected NIH 3T3 cells precipitated with preimmune serum; lane 2, wild-type polyoma-infected cells precipitated with preimmune serum; lane 3, mock-infected cells precipitated with anti-polyoma serum; lane 4, wild-type polyoma-infected cells precipitated with anti-polyoma serum. *b*, Relative PI kinase activity of immunoprecipitates from transformation-defective versus wild-type viral infections. Immunoprecipitates using anti-polyoma serum were made and phosphorylation assays carried out as described below. Lane 1, immunoprecipitate from mock-infected cells; lane 2, immunoprecipitate made from cells infected with the transformation-defective mutant 1387; lane 3, immunoprecipitate from wild-type-infected cells. The position of migration of DPI is indicated. Spots present in all lanes near the top of the TLC are ATP contaminants which vary with the ATP shipment used, and do not depend on addition of either protein or phospholipid to the reaction mix. An additional spot appearing in *a*, lane 4, co-migrates with phosphatidic acid and seems to be due to the presence of a small amount of diacylglycerol in the added phosphatidylinositol.

Methods. NIH 3T3 cells, 24 h post-infection, were washed with cold phosphate-buffered saline, rinsed with 137 mM NaCl, 20 mM Tris pH 8, 1 mM MgCl₂, 1 mM CaCl₂ (buffer A), and lysed in buffer A containing 10% glycerol, 1% NP40, and 1 mM phenylmethylsulphonyl fluoride at 4°C for 20 min. Insoluble material was removed by centrifugation at 4°C for 10 min at 10,000g. The supernatants were incubated with 2 µl of rabbit anti-T serum (D. C. Pallas, M. E. Mahoney, D.R.K. and T.M.R., unpublished) or preimmune serum for 15 min at 25°C. Sepharose C14B-protein A (Sigma) was added, and incubation continued for an additional 15 min. The immunoprecipitates were collected and washed once with cold phosphate-buffered saline, twice with 0.5 M LiCl, 0.1 M Tris pH 7.4, and finally with 10 mM Tris, 100 mM NaCl, 1 mM EDTA. Immunoprecipitates were then assayed for phosphatidylinositol phosphorylation activity: 1 mg ml⁻¹ PI (Avanti) was dispersed by sonication for 15 min in 5 mM HEPES; the dispersed PI was preincubated at 4°C with the immunoprecipitate for 20 min at a final concentration of 0.2 mg PI ml⁻¹. After preincubation, the phosphorylation reaction was started by addition of 20 µCi [γ -³²P]ATP and MgCl₂ to final concentrations of 50 µM ATP and 5 mM MgCl₂ in a volume of 50 µl. The reaction mixture was incubated for 20 min at 25°C and terminated by addition of 100 µl of 1 M HCl. (Less than 10% of the ATP was consumed during the incubation as judged by chromatography on polyethyleneimine-impregnated cellulose in 2 M formate pH 3.5.) Phospholipids were then immediately extracted with 200 µl of CHCl₃/MeOH (1:1). The organic phase was washed once with 80 µl of MeOH/HCl (1:1) and spotted onto a silica gel 60 TLC plate (Merck) pretreated with 1% potassium oxalate. Phosphorylated products were separated by TLC in a CHCl₃/MeOH/4 M NH₄OH (9:7:2) developing solvent for 1 h and visualized by autoradiography. Unlabelled phospholipid standards were included and were visualized by exposure to iodine vapour. Identity of products was also confirmed by two-dimensional chromatography with cold standards; after chromatography in the first dimension in the system described above, plates were developed in the second dimension in CHCl₃/MeOH/CH₃COOH/H₂O (50:39:1:10) (not shown).

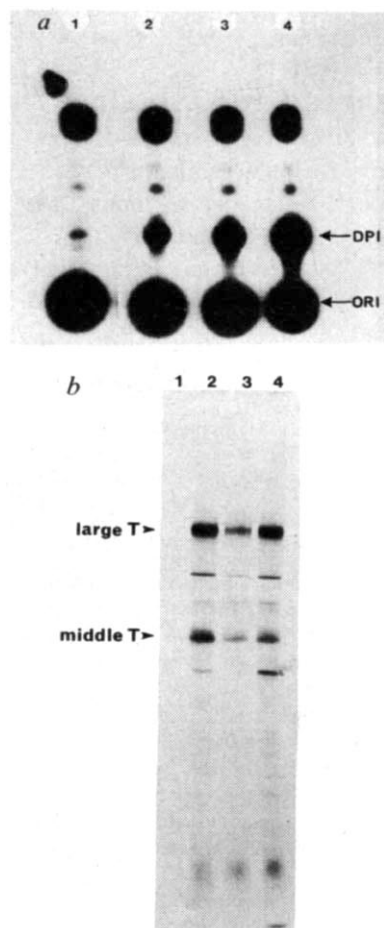


Fig. 2 Comparison of PI kinase activity and middle-T protein in immunoprecipitates from cells infected with wild-type and py1178T mutant of polyoma. NIH 3T3 cells were infected and immunoprecipitations and kinase reactions were performed as in Fig. 1 legend. The cells were labelled with $100 \mu\text{Ci ml}^{-1}$ ^{35}S -methionine for 3 h before the immunoprecipitation. **a**, Autoradiogram for the PI kinase assay. Lane 1, mock-infected cells precipitated with anti-T serum; lane 2, py1178T-infected cells precipitated with anti-T serum; lane 3, cells infected with wild-type virus at 1/10th the py1178T virus multiplicity of infection; lane 4, cells infected with wild-type virus at the same multiplicity of infection as py1178T. **b**, Autoradiograph of ^{35}S -methionine-labelled proteins in the immunoprecipitates separated by SDS-polyacrylamide gel electrophoresis (10% acrylamide, procedure of Laemmli³³). The order of lanes is the same as in **a**.

kinase activity above background, this activity was reduced 5–10-fold relative to wild-type immunoprecipitates when infected cells were making comparable levels of middle-T, measured by incorporation of ^{35}S -methionine (Fig. 2b).

Cellular src (pp60^{c-src}) is associated with polyoma middle-T in immunoprecipitates from infected cells, suggesting that tyrosine phosphorylation of middle-T is catalysed by pp60^{c-src} (ref. 3). Immunoprecipitates of pp60^{c-src} were prepared from normal or polyoma-infected NIH 3T3 cells using the anti-pp60^{c-src} monoclonal antibody GD11 (ref. 34). The immunoprecipitates from polyoma-infected cells (Fig. 3, lanes 11, 12) contained a PI kinase activity ~10-fold above controls (Fig. 3, lanes 5, 6). However, immunoprecipitates of pp60^{c-src} from uninfected cells (Fig. 3, lanes 7, 8) or cells infected with py1387T (not shown) showed no detectable increase in PI kinase activity above controls. In five separate experiments, the pp60^{c-src} immunoprecipitates from polyoma-infected cells showed a 5–20-fold stimulation in PI kinase activity compared with uninfected controls. There was no significant difference in the amounts of pp60^{c-src} in immunoprecipitates from polyoma-infected cells and

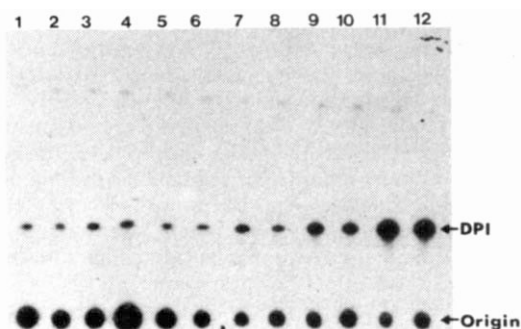


Fig. 3 PI kinase activity of immunoprecipitates made with anti-pp60^{c-src} monoclonal antibody. Viral infections and immunoprecipitations were performed as described in Fig. 1 legend, except that supernatants were incubated with 3 μl of the anti-pp60^{c-src} monoclonal antibody, GD11 (ref. 34), or an equal volume of rabbit preimmune serum for 30 min at 4 °C. PI kinase assays and product separations were performed as in Fig. 1. Lanes 1–6 are immunoprecipitates with control serum and lanes 7–12 are immunoprecipitates with GD11. Lanes 1, 2, 7 and 8 are from uninfected 3T3 cells; lanes 3, 4, 9 and 10 are from 3T3 cells infected with 1 plaque-forming unit per cell of wild-type polyoma virus; lanes 5, 6, 11 and 12 are from 3T3 cells infected with 10 plaque-forming units per cell of wild-type polyoma virus.

Table 1 Relative transformation abilities and PI kinase activities associated with middle-T mutants

Polyoma mutant	PI kinase activity	Transformation competence (ref.)	Association with pp60 ^{c-src} (ref.)
RA (wild type)	100	++(29)	+(3, 4, 24)
Mock infected	7	—	—
py1387T	8	—(20)	+—/(31)
NG59	10	—(25)	—(32)
dl45	165	++(30)	+(24, 31)
py1178T	23	+/(22, 23)	+(31)

3T3 cells were either mock infected or infected with different polyoma mutants. Immunoprecipitations and kinase assays were carried out as described in Fig. 1 legend. For quantitation of PI kinase activity, the radioactive spot corresponding to DPI was cut out, and the silica gel was scraped into a scintillation vial and counted by liquid scintillation spectrometry. PI kinase activities are expressed as % of wild-type activity in immunoprecipitates where comparable amounts of the mutant middle-T protein to wild-type middle-T protein were present as judged by ^{35}S -methionine label. The values presented are the average of at least three separate experiments, except for dl45, where the results of a single experiment are presented. References for the ability of the mutants to cause transformation and to associate with pp60^{c-src} are given in parentheses.

uninfected cells, in agreement with results of Bolen *et al.*²⁴. Thus, middle-T increases the PI kinase activity of pp60^{c-src} immunoprecipitates without increasing the amount of pp60^{c-src}.

Anti-T immunoprecipitates from cells infected with wild-type polyoma virus have at least 20-fold higher PI kinase activity than those from uninfected cells. This activity is correlated with the transforming ability of the virus. Non-transforming mutants NG59 (ref. 25) and py1387T (ref. 20) do not cause such an increase. As py1387T encodes normal large-T and small-T antigens, middle-T must be responsible for the associated PI kinase activity. py1178T has an intermediate phenotype. Its ability to transform F111 cells is severely impaired²³, but DNA containing the mutant sequence transforms Rat 1 cells normally²². Its PI kinase activity, like its transforming ability, is intermediate between those of wild-type and NG59 or py1387T. Like the pp60^{v-src}, some DPI kinase activity and diacylglycerol kinase activity is also associated with the middle-T immunoprecipitates, but these activities are at least fivefold lower than the PI kinase activity when compared at identical substrate and protein concentration (data not presented).

Parallel results were observed when the PI kinase experiments were carried out using anti-pp60^{C-src} instead of anti-T serum. Background PI kinase activity made it difficult to decide whether pp60^{C-src} from uninfected cells has PI kinase activity. Although the simplest model suggests that pp60^{C-src} has a PI kinase activity which is activated by association with middle-T, the possibility that middle-T or an uncharacterized cellular protein associated with middle-T catalyses the PI phosphorylation cannot be excluded. Bolen *et al.*²⁴ have shown recently that pp60^{C-src} immunoprecipitates have enhanced tyrosine kinase activity towards casein. Like pp60^{V-src} (ref. 6) and pp68^{V-ros} (ref. 7), the middle-T/pp60^{C-src} complex may have broad specificity towards protein and lipid substrates. In our hands, the rate of phosphorylation of PI and the model substrate enolase are comparable, giving no clue to the relative importance of the two phosphorylation reactions.

The products of the phosphorylation reactions, phosphatidylinositol diphosphate and triphosphate, have themselves been implicated in the regulation of several membrane-bound enzymes, including the Ca²⁺-ATPase^{10,11} and a nuclear membrane RNA-dependent ATPase implicated in RNA transport^{12,26,27}. Because polyphosphoinositides are the preferred substrates for phospholipase C²⁸, the increased PI kinase activity could raise cellular levels of the breakdown products diacylglycerol and inositol 1,4,5-trisphosphate; the latter compound regulates Ca²⁺ release from internal stores⁸ and the former activates protein kinase C¹⁴. Increased levels of these potential second messengers could provide the pleiotropic response expected for transformation. Characterization of the effects of the middle-T antigen on *in vivo* PI metabolism will be required to confirm and define the physiological significance of this activity.

This work was supported by NIH grants GM28538 (L.C.), CA34722 (B.S.) and CA30002 (T.R.). L.C. and B.S. are Established Investigators of the American Heart Association. M.W. is a NSF Predoctoral Fellow. D.K. is a predoctoral trainee supported by National Service Award GM07196-09.

Received 27 November 1984; accepted 5 March 1985.

- Smith, A. E. & Ely, B. K. *Adv. viral Oncol.* **3**, 3-87 (1983).
- Schaffhausen, B. S. *CRC Rev. Biochem.* **13**, 215-269 (1983).
- Courtneidge, S. & Smith, A. E. *Nature* **303**, 435-439 (1983).
- Courtneidge, S., Oostra, B. K. & Smith, A. E. *Cancer Cells Vol. 2*, 123-132 (Cold Spring Harbor Laboratory, New York, 1984).
- Sefton, B., Hunter, T., Beemon, K. & Eckhart, W. *Cell* **20**, 807-816 (1980).
- Sugimoto, Y., Whitman, M., Cantley, L. & Erikson, R. *Proc. natn. Acad. Sci. U.S.A.* **81**, 2117-2121 (1984).
- Macara, I. G., Marinetti, G. V. & Balduzzi, P. C. *Proc. natn. Acad. Sci. U.S.A.* **81**, 2728-2732 (1984).
- Berridge, M. *Biochem. J.* **220**, 345-360 (1984).
- Michell, R. *Phil. Trans. R. Soc. B296*, 123-137 (1981).
- Penniston, J. T. *Ann. N.Y. Acad. Sci.* **402**, 296-300 (1982).
- Carafoli, E. & Zurini, M. *Biochim. biophys. Acta* **683**, 279-301 (1982).
- Smith, C. D. & Wells, W. W. *J. biol. Chem.* **259**, 11890-11894 (1984).
- Lipsky, J. J. & Lietman, P. S. *Antimicrob. Ag. Chemother.* **18**, 532-535 (1980).
- Nishizuka, Y. *Nature* **308**, 693-698 (1984).
- Whitman, M., Epstein, J. & Cantley, L. *J. biol. Chem.* **259** (in the press).
- Streb, H., Irvine, R. F., Berridge, M. J. & Schulz, I. *Nature* **306**, 67-69 (1983).
- Joseph, S., Thomas, A. P., Williams, R. J., Irvine, R. F. & Williamson, J. R. *J. biol. Chem.* **259**, 3077-3081 (1984).
- Burgess, G. M. *et al. Nature* **309**, 63-66 (1984).
- Kaplan, D. R. *et al. Molec. cell. Biol.* (submitted).
- Carmichael, G. G., Schaffhausen, B. S., Dorsky, D. I., Oliver, D. B. & Benjamin, T. L. *Proc. natn. Acad. Sci. U.S.A.* **79**, 3579-3583 (1982).
- Benjamin, T. L., Carmichael, G. G. & Schaffhausen, B. S. *Cold Spring Harb. Symp. quant. Biol.* **44**, 263-273 (1980).
- Oostra, B. A., Harvey, R., Ely, B. K., Markham, A. F. & Smith, A. E. *Nature* **304**, 456-459 (1983).
- Carmichael, G., Schaffhausen, B., Mandel, G., Liang, T. & Benjamin, T. *Proc. natn. Acad. Sci. U.S.A.* **81**, 679-683 (1984).
- Bolen, J. B. *et al. Cell* **38**, 767-777 (1984).
- Benjamin, T. L. *Proc. natn. Acad. Sci. U.S.A.* **73**, 394-399 (1970).
- Smith, C. C. & Wells, W. W. *J. biol. Chem.* **258**, 9368-9373 (1983).
- Seyfred, M. A. & Wells, W. W. *J. biol. Chem.* **259**, 7659-7665 (1984).
- Wilson, D. B., Bross, T. E., Hofmann, S. L. & Majerus, P. W. *J. biol. Chem.* **259**, 11718-11724 (1984).
- Feunteun, J., Sompayrac, L., Fluck, M. & Benjamin, T. *Proc. natn. Acad. Sci. U.S.A.* **73**, 4169-4173 (1976).
- Bendig, M., Thomas, T. & Folk, W. *J. Virol.* **33**, 1215-1220 (1980).
- Bockus, B., Roberts, T., Parsons, S. & Schaffhausen, B. *Molec. cell. Biol.* (submitted).
- Courtneidge, S. & Smith, A. E. *EMBO J.* **3**, 585-591 (1984).
- Laemmli, U. K. *Nature* **227**, 680-685 (1970).
- Parsons, S. J., McCarty, D. J., Ely, C. M., Benjamin, D. C. & Parsons, J. T. *J. Virol.* **51**, 272-282 (1984).

Primary structure of the α -subunit of transducin and its relationship to *ras* proteins

Tsutomu Tanabe*, Toshihide Nukada*†, Yoshiaki Nishikawa*, Katsumori Sugimoto*, Harukazu Suzuki*, Hideo Takahashi*, Masaharu Noda*, Tatsuya Haga†, Arata Ichiyama†, Kenji Kangawa‡, Naoto Minamino‡, Hisayuki Matsuo‡ & Shosaku Numa*

* Department of Medical Chemistry, Kyoto University Faculty of Medicine, Kyoto 606, Japan

† Department of Biochemistry, Hamamatsu University School of Medicine, Hamamatsu 431-31, Japan

‡ Department of Biochemistry, Miyazaki Medical College, Miyazaki 889-16, Japan

A group of membrane-associated guanine nucleotide binding proteins (G-proteins) are essential for transducing signals generated at cell-surface receptors into changes in cellular function and metabolism¹. These proteins are a complex of three subunits designated α , β and γ . The α -subunit is responsible for binding guanine nucleotides and seems to be characteristic of each protein. Transducin, a member of this protein family, mediates visual transduction by coupling the signal of photolysed rhodopsin with activation of a cyclic GMP phosphodiesterase². We have now cloned and sequenced the complementary DNA encoding the α -subunit of bovine retinal transducin and from this we have deduced the complete amino-acid sequence. The transducin α -subunit shares several homologous amino-acid sequences with *ras* gene products. The homologous segments correspond mostly to the regions thought to be involved in the guanine nucleotide binding and GTPase activity of *ras* proteins and to the ADP-ribosylation sites of the transducin α -subunit.

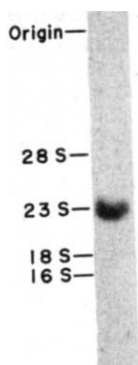
A library of cDNA clones was prepared using poly(A)⁺ RNA from bovine retina as template and the plasmid expression vector pUC8 (refs 3, 4). Screening of the cDNA library⁴ with monoclonal antibodies to the purified α -subunit of porcine retinal transducin yielded two immunologically positive clones from $\sim 8 \times 10^4$ transformants. One of these clones, pT α 108, was found by nucleotide sequence analysis⁵ to contain coding sequences for all six partial amino-acid sequences determined for the transducin α -subunit in the same reading frame (see below). To clone a longer cDNA sequence, we constructed another cDNA library derived from poly(A)⁺ RNA from bovine retina, using the plasmid vector developed by Okayama and Berg⁶. Screening of this library⁷ with a probe derived from the 5'-terminal region of the cDNA insert of clone pT α 108 yielded 61 hybridization-positive clones from $\sim 6 \times 10^5$ transformants. From 36 randomly selected clones, we chose clone pT α 13 for further study. It contained the whole cDNA sequence carried by clone pT α 108 apart from the 3'-terminal 50-nucleotide sequence. The additional sequence in clone pT α 108 seems to have resulted from a possible error during the construction of the cDNA expression library or DNA replication because the sequence was not present in any of the three other clones tested derived from the Okayama-Berg library.

Figure 1 shows the 2,446-nucleotide sequence (excluding the poly(dA) tract) of the cDNA encoding the α -subunit of bovine transducin, determined using clones pT α 13 and pT α 108. The reading frame corresponding to the six partial amino-acid sequences (amino acids 43-47, 83-96, 103-112, 139-157, 177-188 and 301-309) determined by peptide analysis (Fig. 3) was used to deduce the primary structure of the transducin α -subunit (Fig. 1). The translation initiation site was assigned to the methionine codon at nucleotides 1-3 because this is the first in-frame ATG triplet that occurs downstream of the nonsense codon TAG (nucleotide residues -81 to -79) and because the

Fig. 1 Nucleotide sequence of the cDNA for the bovine transducin α -subunit. Nucleotide residues are numbered in the 5' to 3' direction, beginning with the first residue of the ATG triplet encoding the initiating methionine, and the nucleotides on the 5' side of residue 1 are indicated by negative numbers; the number of the nucleotide residue at the right-hand end of each line is given. The deduced amino-acid sequence of the transducin α -subunit is shown above the nucleotide sequence, and amino-acid residues are numbered beginning with the initiating methionine. Nucleotide residue 2,309 in the cDNA insert of clone pT α 13 is followed by a poly(dA) tract, which is connected with the vector DNA sequence^{6,35}. The AATAAA sequence³⁶ is found 15 nucleotides upstream from the polyadenylation site. Nucleotides 840 (T), 1,137 (G) and 1,336 (G) have been taken from pT α 13 (carrying nucleotides -137 to 2,309), whereas the corresponding residues of pT α 108 (carrying nucleotides -30 to 1,453) are C, A and A, respectively; the difference in residue 840 results in no amino-acid substitution.

Methods. Total RNA was extracted³⁷ from bovine retinas and poly(A)⁺ RNA was isolated by oligo(dT)-cellulose chromatography³⁸. The procedures used for the construction of the cDNA expression library and for the immunological screening of transformants were essentially as described previously³⁹. Using 34 μ g of poly(A)⁺ RNA, 805 ng of cDNA was obtained. The screening was carried out by incubation with a mixture of two monoclonal antibodies to the porcine transducin α -subunit (IgG2b isotype), then with ¹²⁵I-protein A (NEN); the monoclonal antibodies were prepared by the hybridoma technique⁴⁰. Clone pT α 108 was thus isolated. For the isolation of clone pT α 13, a cDNA library was constructed by the method of Okayama and Berg⁶, using 7.8 μ g of poly(A)⁺ RNA and 4.2 μ g of the vector-primer DNA. The procedures of transformation and screening were as described previously³⁹; the *Ava*II(-4)/*Ava*II(565) fragment from pT α 108, labelled by nick-translation⁴¹ with [α -³²P]dCTP, was used as hybridization probe.

Fig. 2 Autoradiogram of blot hybridization analysis of bovine retinal RNA with a cDNA probe. Poly(A)⁺ RNA (20 μ g) was analysed by the procedure of Thomas⁹. The hybridization probe used was the *Eco*RI/*Sal*I-excised cDNA insert of clone pT α 108, labelled by nick-translation⁴¹ with [α -³²P]dCTP. The size markers used were bovine and *Escherichia coli* ribosomal RNA⁴⁵. A hybridization-positive band of the same size was observed when the *Ava*II(-4)/*Ava*II(565) fragment derived from pT α 13 was used as probe.



5'-----ACAGCCATTGGATCCG -121
AGTTCTGGGGTCAGAGGGTGTCCAGGGAGGCCAGAGGCTAGCTGAGCCTGGCTCTGGGAAACCCCTGCATCCGGAAGAACCACTACGCGCCCTCTGCTTCACTCTGCGAGGAC -1
1 Met Gly Ala Gly Ala Ser Ala Glu Gly 10 Lys His Ser Arg Glu Leu Glu Lys Lys Leu 20 Lys Glu Asp Ala Glu Lys Asp Ala Arg Thr Val
ATG GGG GCT GGG GCC AGC GCT GAG GAG AAG CAC TCA AAG GAG CTG GAA AAG AAG CTG AAG GAA GAT GCT GAG AAA GAT GCT CGA ACC GTG 90
40 Lys Leu Leu Leu Leu Gly Ala Gly Glu 50 Gly Lys Ser Thr Thr Ile Val Lys Gln Met Lys 110 Ile His Gln Asp Gly Tyr Ser Leu Glu
AAA CTG CTG CTT CTG GGT GCC GGT GAA TCC GGG AAG AGT ACC ATT GTC AAG CAG ATG AAG ATT ATC CAC CAG GAC GGG TAC TCA CTG GAA 180
70 Glu Cys Leu Glu Phe Ile Ala Ile Ile Tyr Gly Asn Thr Leu Gln Ser Ile Leu Ala 110 Val Arg Met Thr Thr Leu Asn Ile Gln
GAG TGT CTT GAG TTC ATT GCC ATC ATC TAT GGC AAC AGC CTA CAG TCC ATC CTG GCC ATT GTG CGC GGC ATT ACC ACA CTC AAG ATC CAG 270
100 Tyr Gly Asp Ser Ala Ser Ala Ser Asp Ala Arg Lys Leu Met His Met Ala Asp Thr 110 Glu Glu Gly Thr Met Pro Lys Glu Met Ser
TAC GGA GAC TCT GCG CCG CAG CAG GAC GAC GCC CGA AAG CTG ATG CAC ATG GCA GAC ACC ATC GAG GAG GGC ACG ATG CCC AAG GAG ATG TCA 360
130 Asp Ile Ile Gln Arg Leu Trp Lys Asp 160 Gly Ile Gln Ala Cys Phe Asp Arg Ala 140 Ser Glu Tyr Gln Leu Asn Asp Ser Ala Gly Tyr
GAC ATC ATC CAG CCG CTG TGG AAG GAC TCC GGT ATC CAG GCC TGT TTC GAC CGA GCC TCA GAG TAC CAG CTC AAC GAC TCT GCT GGC TAC 450
170 Tyr Leu Ser Asp Leu Glu Arg Glu Val Thr 190 Pro Gly Tyr Val Pro Thr Glu Gln Asp 210 Val Leu Cys Ser Arg Val Lys Thr Thr Gly Ile
TAT CTC TCA GAC CTG GAG CCG CTG GTA ACC CGG GAG TAC GTG CCC ACT GAA CAG GAT GTG CTG CCG TCC CGT GTC AAG ACC ACG GGT ATC 540
230 Ile Glu Thr Gln Phe Ser Phe Lys Asp 250 Lys Asn Phe Arg Met Phe Asp Val Gly Gly 270 Gln Arg Ser Glu Arg Lys Lys Trp Ile His Cys
ATT GAG ACG CAG TTC TCC TTC AAG GAC CTC AAC TTT CCG ATG TTC GAT GTG GGC GGG CAG CCG TCA GAG CCG AAG AAG TGG ATC CAC TGC 630
290 Phe Glu Gly Val Thr Cys Ile Ile Phe 310 Ala Ala Leu Ser Ala Tyr Asp Met Val 330 Leu Val Glu Asp Asp Glu Val Asn Arg Met His
TTC GAG GGG GTG ACC TGC ATC ATC TTC ATC CCG GCG CCG CAG GCC TAC GAC ATG GTG CTG GTG GAA GAC GAC GAA GTG AAC CGC ATG CAC 720
370 Glu Ser Leu His Leu Phe Asn Ser Ile 390 Asn His Arg Tyr Phe Ala Thr Thr Ser 410 Ile Val Leu Phe Leu Asn Lys Lys Asp Val Phe
GAG AGC CTG CAC CTG TTC AAC AGT ATC TGC AAC CAC CAG CAC TAC TCC GCC ACC ACG TCC ATC ATC CAG TTT CTC AAC AAG AAG GAC GTC TTC 810
470 Ser Glu Lys Ile Lys Lys Ala His Leu 490 Ile Cys Phe Pro Asp Tyr Asn Gly Pro 510 Asn Thr Tyr Glu Asp Ala Gly Asn Tyr Ile Lys
TCG GAG AAG ATC AAA AAG GCG CAC CTT AGT ATC TGC TTT CCG GAC TAC AAC GGG CCC AAC ACG TAT GAG GAC GCC GGC AAT TAC ATC AAG 900
570 Val Gln Phe Leu Glu Leu Asn Met Arg 590 Asp Val Lys Glu Ile Tyr Ser His Met 610 Cys Ala Thr Asp Thr Gln Asn Val Lys Phe
GTG CAA TTC CTT GAG CTC AAC ATG CGA CGC GAC GTG AAG GAG ATC TAT TCC CAC ATG ACA TGC GCC ACC GAC ACG CAG AAC GTC AAG TTT 990
670 340
Val Phe Asp Ala Val Thr Asp Ile Ile 690 Lys Glu Asn Leu Lys Asp Cys Gly Leu Phe
GTC TTC GAC GCT GTC ACC GAC ATC ATC ATC AAG GAG AAC CTC AAA GAC TGC GGG CTC TTC TGA GGTGCTGAGCTCATGCTCCCTGAGACCTCGAGC 1089
730 CCTTGACACCTTGTAGCCCAATGTCATGACCTATCAGTCCCCAGGACTCCGGGCTCCAGCCTGTGGCCCCACAGCCACACACTCAAAGCCCTAAACCTGTAGCTGTGAGGCC 1209
790 CTATAACCCCTCCCTCGTGGCTCCAGGATTCAGGCTCCGAGAGTCCAATGCCCGAGCCAGCCAGCACTGCCCTTCAGCGCTGGCTGCATCGGCCCTCCAGACCTACCCAGCCA 1329
850 GCCCCAGCTAGAAGCAAGCAGGACTTGGGGCAGCTACAGCTCAGGTGACTCAGCAATACCTCTACTGACCAATCTCTGCTCTCTCTGCCCCAGGAGGCCATTACTCAGCTGGTGA 1449
910 GTCTGGGTTGACCAACAGCCCTCCCTCCAGAGCTTATAGAGGGAGGGGGCAGGCTGAGGCCCTTCTGAGCAGCCCTTCTGCTTACCATCAGCCCTTACGCTTACCACCTC 1569
970 CATGTGACAGGCTCTGATGCCATCTGCCCGAGTGGCAGTACTGCTGCCCTGCCAGCAGATTGAGGAGTCCAAAGTTCACAGCCAGCTCTGCGCTGTGACGCTTGTATGGAGCAGCTTAGT 1689
1030 GAGTCAGGGGACAGCCTGGGGCTCTGGTAGCAGGAAGAGACTCAAGCTGGGAACCTCCCGTACCTACCCCTCCCGCCAGAGGCTCTGCCCCAGCCGCTCTGGACACCAAGAGTCATG 1809
1090 TCCTACTCCCTCCAGCCCGGCCTCTAGATCAGCCACTCTGTGAGGGAGGGTACTGTGACACCACTTGTGGCCAAATGTCACACTGCTCTGAGGGGGTGGTGGGAGCAGAGTCCG 1929
1150 GGTCCATCATGATGAGCCAGGACAGGATGTGAGACCGGAGGAGGAGCAGATTGTCGCCAGAGTGGTCTGCTCAGGAGGGGTCAAAGCAAAATCATGTGACCCCTTGTCCCTGCC 2049
1210 AGCAGCTTTTCTCACCCTTCCCATCAGGGCCCTGGTAGGAAAAAAGATCATGCTGAACCACTCAGGAGCAAAAGCCCATGTCCCTTACCTTGTCTCCCACTTCTCTCATCAAGT 2169
1270 ACCTAGTCTTGTGCTGGCCATGGCCGTAGCCCGTGTGGAGTCAAGGGCAAGGAACATCCGCTAACCAAGTCCAGGGCAATAATTGCACTGCTTACGCTAGATACACAACTTAGC 2289
AATAAACCTTTTGGCTCAGGC-----3'

4a-f). Interestingly, regions *a*, *b* and *d* of *ras* p21 proteins correspond to the regions considered to be involved in guanine nucleotide binding. Region *a* of p21 corresponds to the sequence surrounding Gly 12; this sequence exhibits homology with the nucleotide binding region of the β -subunit of ATP synthase¹⁴ as well as of several dinucleotide binding enzymes¹⁵. Region *b* of some p21 proteins contains a threonine at position 59 which is autophosphorylated by GTP (refs 11, 16). Furthermore, such proteins show a lower GTP binding affinity than do proteins with alanine at position 59 (ref. 13). Region *d* constitutes part of the homologous segment found in G-proteins¹⁷ which has been suggested to lie adjacent to the nucleotide binding site in elongation factor-Tu¹⁸. Thus, regions *a*, *b* and *d* of the transducin α -subunit may also contribute to guanine nucleotide binding. Furthermore, mutations causing amino-acid substitutions at positions 12, 13, 59, 61 or 63 of p21 are known to enhance the transforming potential of *ras* genes¹⁹⁻²⁸ and p21-associated GTPase activity is selectively impaired by an amino-acid substitution at position 12 (ref. 13). Thus, region *a* of the transducin α -subunit, like that of *ras* proteins, may be involved in GTPase activity. Region *c* of the transducin α -subunit contains the tetrapeptide sequence Ser-Arg-Val-Lys, which has been identified as the site that is ADP-ribosylated by cholera toxin²⁹. Region *f* represents the carboxy-terminal tetrapeptide sequence of both the transducin α -subunit and the *ras* proteins (except for the YP2 product) and in the transducin α -subunit, constitutes part of the peptide carrying the site that is ADP-ribosylated by islet-activating protein³⁰. It has been proposed that the conserved cysteine in this region is involved in the post-translational events leading to the biologically active membrane-associated form of

second ATG triplet encodes amino acid 49, which is preceded by one of the peptide sequences determined. A translation termination codon (TGA) occurs in-frame after the 350th codon specifying phenylalanine. Thus, we conclude that the bovine transducin α -subunit is composed of 350 amino-acid residues (including the initiating methionine) and has a calculated relative molecular mass of 39,964, which agrees with the reported value^{2,8}. Blot hybridization analysis⁹ of bovine retinal poly(A)⁺ RNA with cDNA probes showed a hybridization-positive RNA species of ~2,900 nucleotides (Fig. 2).

The *ras* gene products, like the α -subunits of transducin and related proteins, are membrane-associated proteins that bind guanine nucleotides and have GTPase activity¹⁰⁻¹³. Comparison of the amino-acid sequences of the transducin α -subunit and the *ras* protein family reveals several homologous regions (Fig.

p21 (ref. 31). Thus, the regions conserved between the transducin α -subunit and the *ras* proteins seem to correspond to the functional regions of the two groups of G-proteins.

Analysis of the amino-acid sequence of the transducin α -subunit reveals two highly hydrophobic regions with predicted secondary structure^{32,33} which corresponds to continuous

stretches of uncharged amino acids, including many nonpolar residues (residues 65–81 and 213–226). These regions may be involved in hydrophobic interaction with the other transducin subunits or with photolysed rhodopsin or the phosphodiesterase. Alternatively, they may contribute to the association of the protein with the plasma membrane.

Fig. 3 Partial amino-

acid sequence analysis of

the α -subunit of bovine

transducin. **A**, Reverse-

phase HPLC of tryptic

peptides. The tryptic

digest was loaded directly

onto a Chemcosorb 3 μ

C₁₈-H column (8 $\times$

75 mm; Chemco) equi-

librated with 0.1% tri-

fluoroacetic acid, and a

linear gradient of 0–60%

acetonitrile was applied

over 80 min at a flow rate

of 2 ml min⁻¹; a Hitachi

635 HPLC system with a

solvent programmer was

used. The eluate absorbance

at 210 nm was recorded

with a Hitachi 41

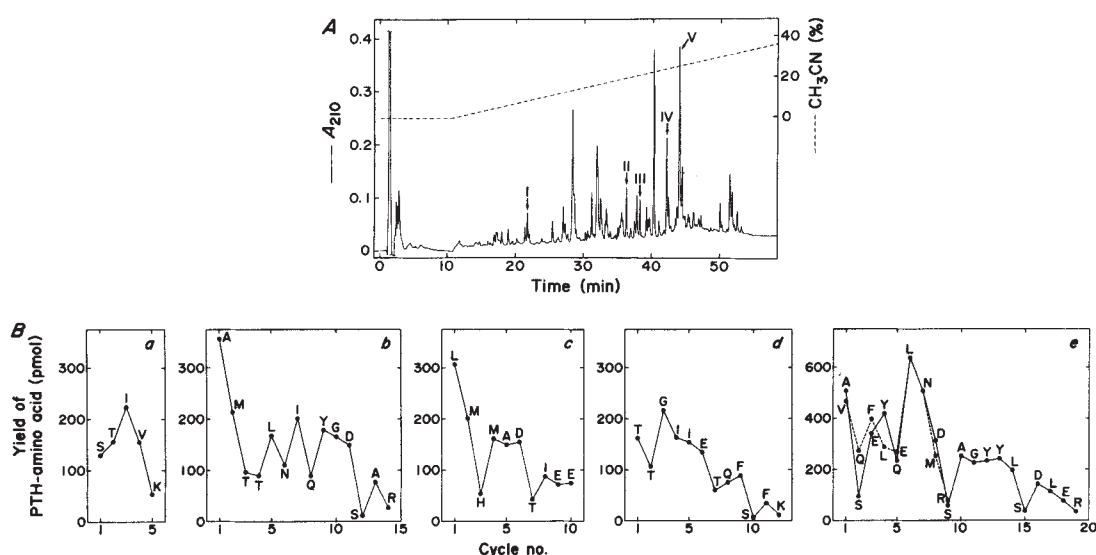
ultraviolet monitor. Five

fractions (I–V) corre-

sponding to absorbance

peaks were collected. **B**, Sequence analysis of peptides. Fractions I (a), II (b), III (c), IV (d) and V (e) were subjected to amino-acid sequence analysis with a gas-phase sequencer (model 470A, Applied Biosystems). Phenylthiohydantoin (PTH)-amino acids were analysed by HPLC at 50 °C using an Ultrasphere ODS column (4.6 $\times$ 250 mm; Altex) with a three-solvent gradient (acetonitrile, water and 40 mM sodium acetate buffer, pH 5.6) at a flow rate of 1 ml min⁻¹. The yield of PTH-amino acids at each cycle of Edman degradation is shown; the one-letter amino-acid notation is used. Fraction V was a mixture of two peptides, whose sequences were assigned as indicated, assuming that leucine and asparagine are shared by the two peptides at the sixth and seventh amino-acid positions, respectively.

Methods. Transducin was partially purified from rod outer segments, prepared from frozen bovine retinas, according to the procedure of Baehr *et al.*⁸. Approximately 20 mg of the partially purified transducin derived from about 1,000 retinas was subjected to electrophoresis on SDS-polyacrylamide gels⁴³ to purify each subunit; electroelution⁴⁴ from the gels yielded 620 μ g, 780 μ g and 300 μ g of the α -, β - and γ -subunits, respectively. Approximately 120 μ g of the purified α -subunit was dialysed against water, lyophilized and dissolved in 830 μ l of 20 mM Tris-acetate buffer, pH 7.8, containing 2 mM CaCl₂. TPCCK-treated trypsin (Worthington) was added at a transducin α -subunit/trypsin ratio of 30 (w/w). After incubation at 37 °C for 12 h, a further identical aliquot of TPCCK-treated trypsin was added, and the incubation was continued for an additional 12 h.



peaks were collected. **B**, Sequence analysis of peptides. Fractions I (a), II (b), III (c), IV (d) and V (e) were subjected to amino-acid sequence analysis with a gas-phase sequencer (model 470A, Applied Biosystems). Phenylthiohydantoin (PTH)-amino acids were analysed by HPLC at 50 °C using an Ultrasphere ODS column (4.6 $\times$ 250 mm; Altex) with a three-solvent gradient (acetonitrile, water and 40 mM sodium acetate buffer, pH 5.6) at a flow rate of 1 ml min⁻¹. The yield of PTH-amino acids at each cycle of Edman degradation is shown; the one-letter amino-acid notation is used. Fraction V was a mixture of two peptides, whose sequences were assigned as indicated, assuming that leucine and asparagine are shared by the two peptides at the sixth and seventh amino-acid positions, respectively.

Methods. Transducin was partially purified from rod outer segments, prepared from frozen bovine retinas, according to the procedure of Baehr *et al.*⁸. Approximately 20 mg of the partially purified transducin derived from about 1,000 retinas was subjected to electrophoresis on SDS-polyacrylamide gels⁴³ to purify each subunit; electroelution⁴⁴ from the gels yielded 620 μ g, 780 μ g and 300 μ g of the α -, β - and γ -subunits, respectively. Approximately 120 μ g of the purified α -subunit was dialysed against water, lyophilized and dissolved in 830 μ l of 20 mM Tris-acetate buffer, pH 7.8, containing 2 mM CaCl₂. TPCCK-treated trypsin (Worthington) was added at a transducin α -subunit/trypsin ratio of 30 (w/w). After incubation at 37 °C for 12 h, a further identical aliquot of TPCCK-treated trypsin was added, and the incubation was continued for an additional 12 h.

a Transducin α (31–53) c-Ha-ras1 (5–27) v-Ha-ras (5–27) c-Ki-ras2 (5–27) v-Ki-ras (5–27) N-ras (5–27) v-Ra-ras (64–86) Dras1 (5–27) Dras2 (5–24) c-ras^{SC}-1 (12–34) c-ras^{SC}-2 (12–34) YP2 (10–32)	b Transducin α (85–101) c-Ha-ras1 (58–73) v-Ha-ras (58–73) c-Ki-ras2 (58–73) v-Ki-ras (58–73) N-ras (58–73) v-Ra-ras (117–132) Dras1 (58–73) Dras2 (55–70) c-ras^{SC}-1 (65–80) c-ras^{SC}-2 (65–80) YP2 (64–79)	c Transducin α (167–178) c-Ha-ras1 (98–106) v-Ha-ras (98–106) c-Ki-ras2 (98–106) v-Ki-ras (98–106) N-ras (98–106) v-Ra-ras (157–165) Dras1 (98–106) Dras2 (95–103) c-ras^{SC}-1 (105–113) c-ras^{SC}-2 (105–113) YP2 (104–112)
d Transducin α (228–233) c-Ha-ras1 (111–116) v-Ha-ras (111–116) c-Ki-ras2 (111–116) v-Ki-ras (111–116) N-ras (111–116) v-Ra-ras (170–175) Dras1 (111–116) Dras2 (108–113) c-ras^{SC}-1 (118–123) c-ras^{SC}-2 (118–123) YP2 (117–121)	e Transducin α (288–295) c-Ha-ras1 (148–155) v-Ha-ras (148–155) c-Ki-ras2 (exon 4A) (148–155) c-Ki-ras2 (exon 4B) (148–155) v-Ki-ras (148–155) N-ras (148–155) v-Ra-ras (207–214) Dras1 (148–155) Dras2 (146–153) c-ras^{SC}-1 (156–163) c-ras^{SC}-2 (156–163) YP2 (154–161)	f Transducin α (347–350) c-Ha-ras1 (186–189) v-Ha-ras (186–189) c-Ki-ras2 (exon 4A) (186–189) c-Ki-ras2 (exon 4B) (185–188) v-Ki-ras (186–189) N-ras (186–189) v-Ra-ras (245–248) Dras1 (186–189) Dras2 (184–187) c-ras^{SC}-1 (306–309) c-ras^{SC}-2 (319–322) YP2 (206)

Fig. 4 Amino-acid sequence homology in different regions (a–f) between the transducin α -subunit and *ras* proteins. The sequences of the *ras* gene products have been taken from the literature: c-Ha-ras1 (ref. 46); v-Ha-ras (ref. 47); c-Ki-ras2 (ref. 25); v-Ki-ras (ref. 48); N-ras (ref. 26); v-Ra-ras (ref. 49); Dras1 and Dras2 (ref. 50); c-ras^{SC}-1 (refs 51, 52); c-ras^{SC}-2 (ref. 52); YP2 (ref. 53). The one-letter amino-acid notation is used. Amino-acid residues are numbered beginning with the initiating methionine of the respective polypeptides, and the residues constituting each aligned sequence are indicated by their numbers. Identities or favoured substitutions, as compared with the residues of the transducin α -subunit, are boxed. Favoured substitutions are defined as pairs of residues belonging to one of the following groups: S, T, P, A and G; N, D, E and Q; H, R and K; M, I, L and V; F, Y and W (ref. 54). Gaps (–) have been inserted to achieve maximum homology. In e and f, both the sequences encoded by exon 4A and exon 4B of c-Ki-ras2 are given.

Partial amino-acid sequences of the bovine transducin α -subunit reported recently by Hurley *et al.*³⁴ correspond, with some undetermined residues, to residues 19–51, 205–226 and 311–350 of our deduced amino-acid sequence. These authors have also noted the sequence homology between the transducin α -subunit and *ras* proteins in the region surrounding Gly 12 of p21.

We thank Drs Paul Berg and Hiroto Okayama for donating their high-efficiency cloning system and Dr Takashi Miyata and Mr Hiroyuki Toh for computer analysis. This investigation was supported in part by research grants from the Ministry of Education, Science and Culture of Japan, the Mitsubishi Foundation and the Japanese Foundation of Metabolism and Diseases.

Received 2 January; accepted 21 February 1985.

1. Gilman, A. G. *Cell* **36**, 577–579 (1984).
2. Stryer, L. *Cold Spring Harb. Symp. quant. Biol.* **48**, 841–852 (1983).
3. Vieira, J. & Messing, J. *Gene* **19**, 259–268 (1982).
4. Helfman, D. M., Feramisco, J. R., Fiddes, J. C., Thomas, G. P. & Hughes, S. H. *Proc. natn. Acad. Sci. U.S.A.* **80**, 31–35 (1983).
5. Maxam, A. M. & Gilbert, W. *Meth. Enzym.* **65**, 499–560 (1980).
6. Okayama, H. & Berg, P. *Molec. cell. Biol.* **2**, 161–170 (1982).
7. Hanahan, D. & Messelson, M. *Gene* **10**, 63–67 (1980).
8. Baehr, W., Morita, E. A., Swanson, R. J. & Applebury, M. L. *J. biol. Chem.* **257**, 6452–6460 (1982).
9. Thomas, P. S. *Proc. natn. Acad. Sci. U.S.A.* **77**, 5201–5205 (1980).
10. Scolnick, E. M., Papageorge, A. G. & Shih, T. Y. *Proc. natn. Acad. Sci. U.S.A.* **76**, 5355–5359 (1979).
11. Shih, T. Y., Papageorge, A. G., Stokes, P. E., Weeks, M. O. & Scolnick, E. M. *Nature* **287**, 686–691 (1980).
12. Willingham, M. C., Pastan, I., Shih, T. Y. & Scolnick, E. M. *Cell* **19**, 1005–1014 (1980).
13. McGrath, J. P., Capon, D. J., Goeddel, D. V. & Levinson, A. D. *Nature* **310**, 644–649 (1984).
14. Gay, N. J. & Walker, J. E. *Nature* **301**, 262–264 (1983).
15. Wierenga, R. K. & Hol, W. G. J. *Nature* **302**, 842–844 (1983).
16. Shih, T. Y., Stokes, P. E., Smythers, G. W., Dhar, R. & Oroszlan, S. *J. biol. Chem.* **257**, 11767–11773 (1982).
17. Leberman, R. & Egner, U. *EMBO J.* **3**, 339–341 (1984).
18. Arai, K. *et al. Proc. natn. Acad. Sci. U.S.A.* **77**, 1326–1330 (1980).
19. Tabin, C. J. *et al. Nature* **300**, 143–149 (1982).

20. Reddy, E. P., Reynolds, R. K., Santos, E. & Barbacid, M. *Nature* **300**, 149–152 (1982).
21. Taparowsky, E. *et al. Nature* **300**, 762–765 (1982).
22. Yuasa, Y. *et al. Nature* **303**, 775–779 (1983).
23. Santos, E., Reddy, E. P., Pulciani, S., Feldmann, R. J. & Barbacid, M. *Proc. natn. Acad. Sci. U.S.A.* **80**, 4679–4683 (1983).
24. Shimizu, K. *et al. Nature* **304**, 497–500 (1983).
25. McGrath, J. P. *et al. Nature* **304**, 501–506 (1983).
26. Taparowsky, E., Shimizu, K., Goldfarb, M. & Wigler, M. *Cell* **34**, 581–586 (1983).
27. Sukumar, S., Nataro, V., Martin-Zanca, D. & Barbacid, M. *Nature* **306**, 658–661 (1983).
28. Fasano, O. *et al. Proc. natn. Acad. Sci. U.S.A.* **81**, 4008–4012 (1984).
29. Van Dop, C., Tsubokawa, M., Bourne, H. R. & Ramachandran, J. *J. biol. Chem.* **259**, 696–698 (1984).
30. Manning, D. R., Fraser, B. A., Kahn, R. A. & Gilman, A. G. *J. biol. Chem.* **259**, 749–756 (1984).
31. Willumsen, B. M., Norris, K., Papageorge, A. G., Hubbert, N. L. & Lowy, D. R. *EMBO J.* **3**, 2581–2585 (1984).
32. Chou, P. Y. & Fasman, G. D. *A. Rev. Biochem.* **47**, 251–276 (1978).
33. Kyte, J. & Doolittle, R. F. *J. molec. Biol.* **157**, 105–132 (1982).
34. Hurley, J. B., Simon, M. I., Teplow, D. B., Robishaw, J. D. & Gilman, A. G. *Science* **226**, 860–862 (1984).
35. Reddy, V. B. *et al. Science* **200**, 494–502 (1978).
36. Proudfoot, N. J. & Brownlee, G. G. *Nature* **263**, 211–214 (1976).
37. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. *Biochemistry* **18**, 5294–5299 (1979).
38. Aviv, H. & Leder, P. *Proc. natn. Acad. Sci. U.S.A.* **69**, 1408–1412 (1972).
39. Noda, M. *et al. Nature* **312**, 121–127 (1984).
40. Köhler, G. & Milstein, C. *Nature* **256**, 495–497 (1975).
41. Weinstock, R., Sweet, R., Weiss, M., Cedar, H. & Axel, R. *Proc. natn. Acad. Sci. U.S.A.* **75**, 1299–1303 (1978).
42. Hewick, R. M., Hunkapiller, M. W., Hood, L. E. & Dreyer, W. J. *J. biol. Chem.* **256**, 7990–7997 (1981).
43. Laemmli, U. K. *Nature* **227**, 680–685 (1970).
44. Hunkapiller, M. W., Lujan, E., Ostrander, F. & Hood, L. E. *Meth. Enzym.* **91**, 227–236 (1983).
45. McMaster, G. K. & Carmichael, G. G. *Proc. natn. Acad. Sci. U.S.A.* **74**, 4835–4838 (1977).
46. Capon, D. J., Chen, E. Y., Levinson, A. D., Seeburg, P. H. & Goeddel, D. V. *Nature* **302**, 33–37 (1983).
47. Dhar, R. *et al. Science* **217**, 934–937 (1982).
48. Tsuchida, N., Ryder, T. & Ohtsubo, E. *Science* **217**, 937–939 (1982).
49. Rasheed, S., Norman, G. L. & Heidecker, G. *Science* **221**, 155–157 (1983).
50. Neuman-Silberberg, F. S., Schejter, E., Hoffmann, F. M. & Shilo, B.-Z. *Cell* **37**, 1027–1033 (1984).
51. DeFeo-Jones, D., Scolnick, E. M., Koller, R. & Dhar, R. *Nature* **306**, 707–709 (1983).
52. Powers, S. *et al. Cell* **36**, 607–612 (1984).
53. Gallwitz, D., Donath, C. & Sander, C. *Nature* **306**, 704–707 (1983).
54. Dayhoff, M. O., Schwartz, R. M. & Orcutt, B. C. in *Atlas of Protein Sequence and Structure* Vol. 5, Suppl. 3 (ed. Dayhoff, M. O.) 345–352 (National Biomedical Research Foundation, Silver Spring, Maryland, 1978).

Translocation of vesicles from squid axoplasm on flagellar microtubules

Susan P. Gilbert, Robert D. Allen & Roger D. Sloboda*

Department of Biological Sciences, Dartmouth College, Hanover, New Hampshire 03755, USA, and Marine Biological Laboratory, Woods Hole, Massachusetts 02543, USA

Directed intracellular particle movement is a fundamental process characteristic of all cells. During fast axonal transport, membranous organelles move at rapid rates, from 1 to $5 \mu\text{m s}^{-1}$, in either the orthograde or retrograde direction along the neurone and can traverse distances as long as 1 m (for reviews, see refs 1–3). Recent studies indicate that this extreme example of intracellular motility can occur along single microtubules⁴, but the molecules generating the motile force have not been identified or localized. It is not known whether the force-transducing 'motor' is associated with the moving particle or with the microtubule lattice. To distinguish between these hypotheses and to characterize the membrane-cytoskeletal interactions that occur during vesicle translocations, we have developed a reconstituted model for microtubule-based motility. We isolated axoplasmic vesicles from the giant axon of the squid *Loligo pealei* as described previously⁵. The vesicles (35–475 nm in diameter) were then added to axonemes of *Arbacia punctulata* spermatozoa that served as a source of microtubules. Axonemes were used because the tubulin subunit lattice of the A-subfibre of a given outer doublet is the same as the subunit lattice of neuronal microtubules along which motility occurs^{6,7}. Moreover, all the microtubules of a single axoneme show the same structural polarity^{8–10}, indicating that the axoneme represents an oriented microtubule substrate. Here we demonstrate that vesicle motility is ATP-dependent, that it is not mediated by the flagellar force-transducing molecule dynein and that the direction of movement is not specified by microtubule polarity.

When axoplasmic vesicles are added to flagellar microtubules in the presence of ATP, the vesicles move along the axonemal microtubules in either direction on a single axoneme. Figure 1a–c shows a vesicle (arrowhead) that has moved to the end of the axoneme and has remained attached on reaching the end. Figure 1d–f shows another vesicle that became associated with the same axoneme 8 minutes later, and the vesicle travelled in the opposite direction to that shown in a–c. The axonemes used in Fig. 1 were prepared from *Arbacia* spermatozoa by dialysis against low ionic strength Tris-EDTA buffer to remove the dynein arms¹¹. SDS-polyacrylamide gel electrophoresis (Fig. 2) demonstrates that the dynein has been removed from the axonemes shown in Fig. 1. (Figure 2d shows the polypeptide profile of the axonemes before Tris-EDTA dialysis; lane e shows these axonemes after the dialysis, whereas lane f shows the proteins removed from the axonemes by the dialysis treatment.) These data demonstrate that the axonemes supporting vesicle motility lack dynein, the flagellar force-transducing molecule.

To examine the vesicle-microtubule interaction illustrated in Fig. 1 at higher resolution, a sample of axonemes and associated vesicles was fixed and prepared for thin-section electron microscopy (Fig. 3). The vesicle in Fig. 3a is in association with an axoneme; b illustrates a representative field of axonemes and a vesicle attached to a microtubule. Figure 3c shows that the vesicle membrane contacts the microtubule by crossbridging filaments. The distance between the crosslinks varies from 14 to 20 nm. Those segments of microtubules that are not in association with a vesicle lack these filamentous crosslinks. The other projections seen on the axonemal microtubules have the periodicity and appearance of radial spokes¹² and are unaffected by the low ionic strength dialysis that removes the dynein arms. Both the crossbridging filaments and the axonemal projections lack the tilt and periodicity (24 nm) expected for dynein. The sample used to generate Fig. 3 was obtained from the same axoneme/vesicle preparation as in Fig. 1. For electron microscopy, however, axonemes and vesicles were combined and fixed in solution. Thus, although Fig. 3 shows apparent inter-

* To whom correspondence should be addressed.

Fig. 1 Video micrograph of two vesicles moving on flagella axonemal microtubules in the absence of flagellar dynein. The sequence in *a-c* demonstrates a vesicle (arrowhead) that became attached to the axoneme at ~2 min after the vesicles were added to the axonemal microtubules. The sequence in *d-f* shows another vesicle (arrowhead) that became attached to the same axoneme 8 min later and moved in the opposite direction. The time (min:s) of each photograph of the video record is indicated. Scale bar, 1.2 μ m.

Methods. Axonemal microtubules were prepared from *Arbacia* spermatozoa and dialysed against 1 mM Tris-HCl pH 8.0, 0.1 mM EDTA, 0.1% 2-mercaptoethanol to remove the dynein¹¹. The axonemes were collected by centrifugation and washed 2-3 times in HEPES buffer (10 mM HEPES pH 7.0, 0.1 M KCl, 5 mM MgSO₄, 0.5 mM EDTA, 1 mM dithiothreitol). Washed axonemes were allowed to settle on a no. 0 coverslip and any unattached axonemes were removed by washing with the HEPES buffer. The preparation was covered with a second coverslip and transferred to the microscope for observation. Axoplasmic vesicles were isolated from the giant axon of the squid *Loligo pealei* using discontinuous sucrose gradient centrifugation as described previously⁵. These vesicles in buffer (0.3 M sucrose, 100 mM KCl, 10 mM MgCl₂, 1 mM EGTA, 1 mM ATP, 1 mM phenylmethylsulphonyl fluoride, 10 mM imidazole, pH 7.4) were added at the edge of the coverslip 'sandwich' containing attached axonemes and washed across the preparation by absorbing buffer with filter paper at the opposite side of the coverslip. Observations of the vesicle addition and of the vesicle movement were made by video-enhanced contrast differential interference contrast microscopy¹⁸.

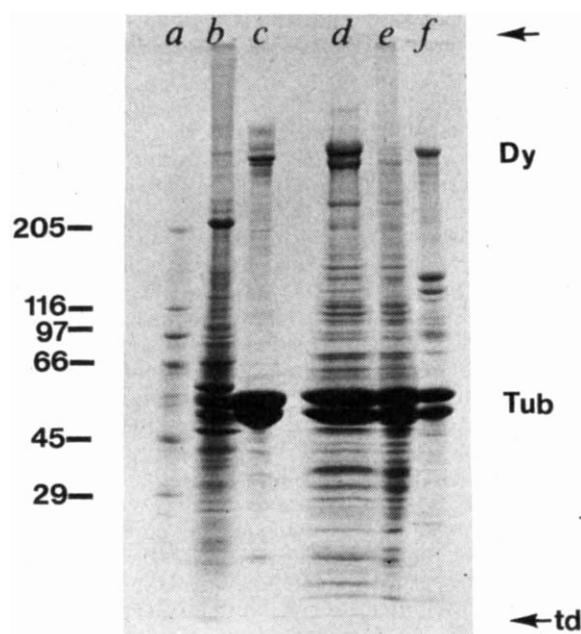
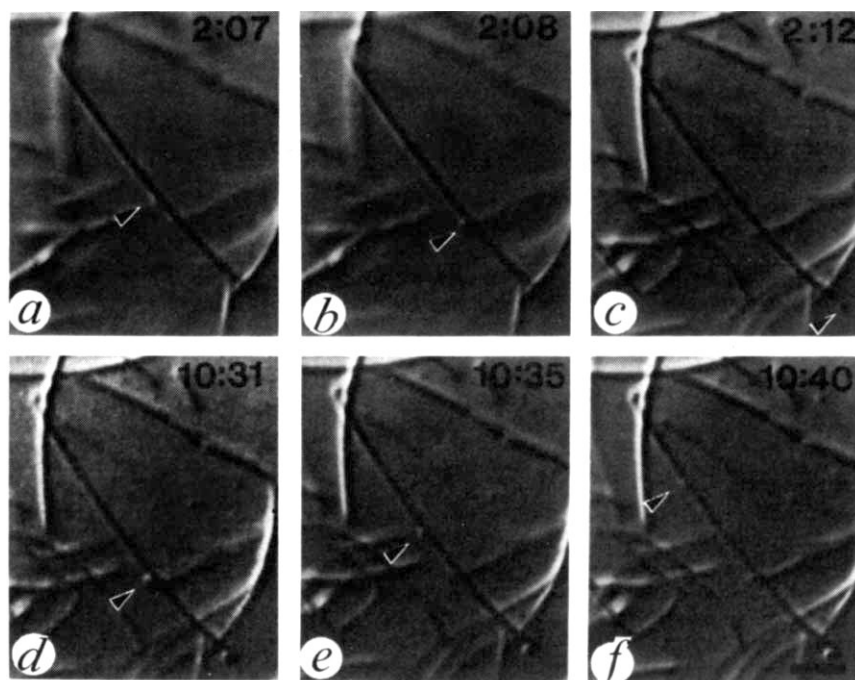


Fig. 2 SDS-polyacrylamide gel electrophoretic analysis of the protein composition of the axonemal microtubule preparation. Lanes *a*, *b* and *c* show various standards: *a*, relative molecular mass markers ($\times 10^{-3}$); *b*, squid axoplasm; *c*, purified mammalian brain microtubules. Lane *d* demonstrates the polypeptide composition of the axoneme preparation before the Tris-EDTA dialysis; lane *e*, the axonemes after dialysis and centrifugation, showing the loss of dynein (Dy) from the axonemal microtubules. (This sample (lane *e*) is from the same preparation as that used as the source of axonemes in Fig. 1.) Lane *f* shows the proteins removed from the axonemes by the dialysis step. Arrows indicate the top and bottom of the 3-15% acrylamide/12-48% urea linear gradient slab gel, using the buffer formulation of Laemmli¹⁹ and stained for protein with Coomassie brilliant blue. Tub, tubulin; td, tracking dye.

action between a moving vesicle and an axoneme, we did not determine whether this vesicle was moving at the time of fixation.

We have found that axoplasmic vesicles move along: (1) outer doublets; (2) outer doublets treated with 0.5 M KCl to remove the outer dynein arms¹³; and (3) axonemes dialysed against Tris-EDTA to remove both the outer and inner dynein arms¹¹, but they do not move along intact flagella (that is, axonemes with the flagellar membrane present) or on glass fibres. Moreover, neither large unilamellar liposomes (100-400 nm in diameter; phosphatidylserine/phosphatidylcholine, 2:1) nor microspheres (200 and 400 nm in diameter) move along axonemes in the buffer that supports vesicle movement. Axonemes without added vesicles never demonstrate particle movement in the presence of ATP.

Rates of vesicle movement on axonemes subjected to various treatments were quantitated from video records⁵. Figure 4 illustrates similar rates of vesicle movement in three different axonemal preparations. The rates in *a* describe vesicles moving on outer doublets. The outer doublets were prepared by treating axonemes with trypsin to hydrolyse the nexin links, followed by reactivation with ATP to cause the microtubule outer doublets to slide relative to one another^{14,15}. This results in the loss of the axonemal 9+2 configuration, but the dynein arms remain intact. The axonemal preparation in Fig. 4*b* was obtained by treating outer doublets (isolated as described for *a*) with 0.5 M KCl to remove the outer dynein arms¹³. The axonemes of Fig. 4*c* were dialysed against low ionic strength Tris-EDTA to remove both the outer and inner dynein arms¹¹.

Vesicles were observed to move in both directions on a single axoneme (Fig. 1). These observations indicate that the polarity of the microtubule did not dictate the directionality of vesicle movement because all the microtubules of a single axoneme have the same polarity⁸⁻¹⁰. Most vesicles do not change their direction of travel once in association with the axoneme, although we observed one vesicle that stopped and changed direction of translocation along the axoneme and some vesicles that changed from one axoneme to another and continued moving. However, the rates of movement were the same both for a vesicle that moved on two different axonemes and for several vesicles on the same axoneme. Furthermore, experiments

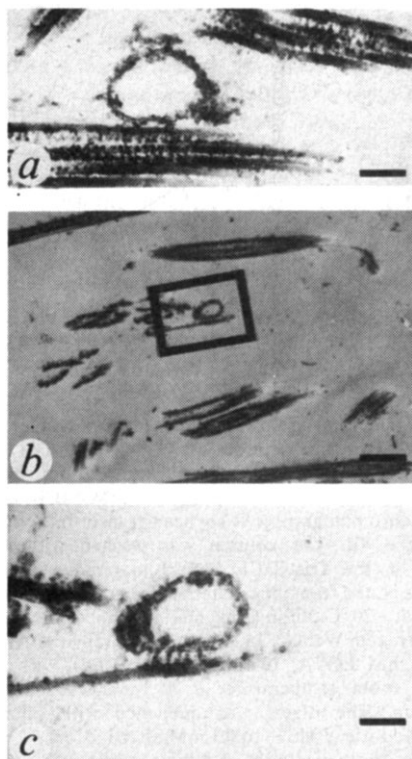


Fig. 3 Transmission electron micrograph illustrating vesicle association with axonemal microtubules. *a*, The vesicle (230×346 nm) illustrated is in contact with an axoneme. *b*, A typical field of axonemes with one vesicle in association with an axonemal microtubule. *c*, Higher magnification of the area enclosed in the rectangle in *b*. The vesicle (180×309 nm) is in association with an axonemal microtubule (24 nm) and seems to be attached by cross-bridging filaments; the spacing between the filaments is 14–20 nm. **Methods.** Vesicles and axonemes were prepared as described in Fig. 1 legend. Vesicles in buffer were added to axonemes and incubated for 5 min to allow vesicles to associate with axonemal microtubules. The preparation was fixed in 2% glutaraldehyde, 1% tannic acid, 1 mM MgSO_4 , 0.1 M sodium phosphate buffer, pH 6.8 at 4 °C and post-fixed in 1% osmium tetroxide at 4 °C. The sample was dehydrated in ethanol, transferred through three changes of propylene oxide and embedded in LX-112 (Ladd Research Industries, Burlington, Vermont). Thin sections (60–70 nm) were stained with saturated aqueous uranyl acetate followed by lead citrate²⁰ and observed with a JEOL 100CX electron microscope at an accelerating voltage of 60 kV. *a*, Scale bar 150 nm; *b*, scale bar 500 nm; *c*, scale bar 100 nm.

designed to test the competency of axonemes to support vesicle motility demonstrate that all axonemes tested support vesicle movement.

The results presented here demonstrate clearly that vesicles from squid axoplasm move on flagellar axonemal microtubules at the same rate in the presence or absence of flagellar dynein. Thus, vesicle motility is not mediated by the axonemal force-generating molecule dynein. In addition, the vesicle motility seems to be a different phenomenon from that studied by Bloodgood *et al.* in which polystyrene microspheres move on *Chlamydomonas* flagella^{16,17}. The ATPase mediating the motility of microspheres on *Chlamydomonas* flagella demonstrates a strict dependence on both the flagellar membrane and Ca^{2+} . In contrast, axoplasmic vesicles do not move on intact flagella (that is, axonemes with membrane in place); furthermore, vesicle motility on axonemal microtubules occurs in the presence of 1 mM EGTA (that is, in the absence of calcium). The results presented here, when taken together with those of Gilbert and Sloboda⁵, suggest that key vesicle surface proteins interact with the microtubule to effect vesicle movement. However, it is possible that there is an as yet uncharacterized ATPase associated

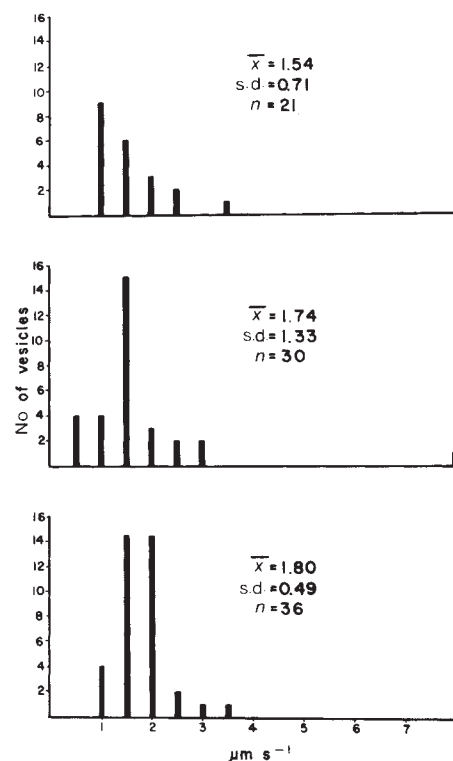


Fig. 4 Rates of vesicle movement on axonemal microtubules. *a*, Rates of vesicle movement occurring on axonemal outer doublet microtubules obtained by treating axonemes with trypsin followed by soybean trypsin inhibitor and reactivation with ATP. This treatment caused the outer doublet microtubules to slide apart out of the normal 9 + 2 configuration of the axoneme¹⁴. *b*, Rates of movement of vesicles on outer doublets obtained as in *a*, followed by treatment with 0.5 M KCl to remove the outer dynein arms¹³. *c*, Rates of movement of vesicles on intact axonemes dialysed against Tris-EDTA to remove the outer and inner dynein arms¹¹. The three mean rates ($\bar{x}$) are not significantly different at a *P* of 0.05 as determined by either Student's *t*-test or Mann-Whitney *U* test. $\bar{x}$, Mean; s.d., standard deviation; *n*, sample size.

with the axoneme that remains bound under conditions that remove flagellar dynein from axonemal microtubules. Alternatively, a soluble axoplasmic ATPase not associated with the membrane may co-purify with the vesicles and mediate vesicle movement. The results reported here do not distinguish between these possibilities, but rather present a rapid *in vitro* motility assay that may be used to evaluate microtubule-dependent motility. This model system will allow vesicle characteristics to be manipulated experimentally to localize and characterize the force-transducing molecules involved in fast axonal transport.

We thank Dr Robert H. Gross for the gift of liposomes. This research was supported by grants to R.D.S. (Muscular Dystrophy Association; NSF PCM 80-21976) and S.P.G. (Sigma Xi Grant-in-Aid and Albert Cass Traveling Fellowship). The microscope was provided by a grant to R.D.A. (Rowland Foundation). S.P.G. is a graduate student at Dartmouth College supported by a R. Mellville Cramer Graduate Fellowship and by NIH-NINCDS grant NS19962 to R.D.A.

Received 31 January; accepted 11 March 1985.

1. Brady, S. T. & Lasek, R. J. *Meth. Cell Biol.* **25**, 365–398 (1982).
2. Grafstein, B. & Forman, D. S. *Physiol. Rev.* **60**, 1167–1283 (1980).
3. Weiss, D. G. (ed.) *Axoplasmic Transport* (Springer, New York, 1982).
4. Schnapp, B. J., Vale, R. D., Sheetz, M. P. & Reese, T. S. *Cell* **40**, 455–462 (1985).
5. Gilbert, S. P. & Sloboda, R. D. *J. Cell Biol.* **99**, 445–452 (1984).
6. Amos, L. A. & Klug, A. J. *Cell Sci.* **14**, 523–559 (1974).
7. Erickson, H. P. *J. Cell Biol.* **60**, 153–167 (1974).
8. Allen, C. & Borisy, G. G. *J. molec. Biol.* **90**, 381–402 (1974).
9. Binder, L. I., Dentler, W. L. & Rosenbaum, J. L. *Proc. natn. Acad. Sci. U.S.A.* **72**, 1122–1126 (1975).

10. Haimo, L. T., Telzer, B. R. & Rosenbaum, J. L. *Proc. natn. Acad. Sci. U.S.A.* **76**, 5759-5763 (1979).
11. Gibbons, I. R. *Archs Biol., Liege* **76**, 317-352 (1965).
12. Warner, F. D. & Satir, P. *J. Cell Biol.* **63**, 35-63 (1974).
13. Gibbons, B. H. & Gibbons, I. R. *J. Cell Sci.* **13**, 337-357 (1973).
14. Summers, K. E. & Gibbons, I. R. *Proc. natn. Acad. Sci. U.S.A.* **68**, 3092-3096 (1971).
15. Summers, K. E. & Gibbons, I. R. *J. Cell Biol.* **58**, 618-629 (1973).
16. Bloodgood, R. A. *J. Cell Biol.* **75**, 983-989 (1977).
17. Bloodgood, R. A., Leffler, E. M. & Bojczuk, A. T. *J. Cell Biol.* **82**, 664-674 (1979).
18. Allen, R. D. & Allen, N. S. *J. Microsc.* **129**, 3-17 (1983).
19. Laemmli, U. K. *Nature* **227**, 680-685 (1970).
20. Reynolds, E. S. *J. Cell Biol.* **17**, 208-211 (1963).

A gelsolin-like Ca^{2+} -dependent actin-binding domain in villin

Paul Matsudaira*, Ross Jakes & John E. Walker

Medical Research Council, Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK

Villin is an actin-binding protein of relative molecular mass (M_r) 95,000 found in the core bundle of microfilaments in brush border microvilli from intestine¹⁻³. In physiological calcium concentrations ($<1 \mu\text{M}$), villin crosslinks actin filaments into bundles. However, in free calcium concentrations ($>1 \mu\text{M}$), villin severs actin filaments into short pieces³⁻⁵. To understand how villin can sever and bundle actin filaments, we are studying the molecular basis of villin-actin binding interactions by identifying important actin-binding domains in villin. Here, we report the purification and preliminary characterization of a 44,000- M_r fragment of villin which contains a calcium-dependent actin-severing activity. In addition, the partial amino-acid sequence from the amino terminus of this fragment reveals homology with a 16-residue region near the amino terminus⁶ of gelsolin, an actin-severing protein found in many cells⁷⁻⁹ and sera¹⁰⁻¹². The sequence homology suggests a common structural basis for the calcium-regulated actin-severing properties shared by villin and gelsolin.

Glenney and co-workers^{13,14} used V-8 protease to cleave villin into a 90,000- M_r amino-terminal fragment (villin core) and an 8,500- M_r carboxy-terminal fragment (villin headpiece) (Fig. 4c). Using electron microscopy, viscometry and pelleting assays, they showed that villin core retained the calcium-dependent actin-severing activity but lost the bundling activity of the intact villin molecule. In comparison, villin headpiece, independent of calcium, could bind but not bundle or sever actin filaments¹³⁻¹⁵. More recently, Hesterberg and Weber^{16,17} identified one non-exchangeable and two exchangeable (with micromolar dissociation constants) calcium-binding sites in villin. One of the exchangeable sites was located in villin headpiece and the other two sites in villin core. We have used trypsin and other proteases to determine whether the calcium-dependent severing activity of villin can be localized to a fragment smaller than villin core. Trypsin cleaved villin into two fragments¹⁸ of M_r 44,000 and 51,000 (named 44T and 51T, respectively, Fig. 1a). The time course of appearance of 44T and 51T (Fig. 1b) shows that both fragments are produced at similar rates and suggests that trypsin cleaves villin near the middle of the molecule. Digests for longer times or at higher trypsin/villin ratios caused further degradation of 51T while 44T remained stable to proteolysis. The instability of 51T relative to 44T explains the lower accumulation of 51T during proteolysis.

Villin fragments which retain calcium-regulated actin-binding activity were identified using the ¹²⁵I-actin gel overlay assay devised by Snabes and co-workers^{19,20}. (They used this assay to identify gelsolin-like proteins in a variety of other cells and tissues.) In our experiments (Fig. 2), the ¹²⁵I-actin probe bound villin and 44T only in the presence of calcium; 51T did not bind actin in either calcium or EGTA. Thus, a calcium-regulated

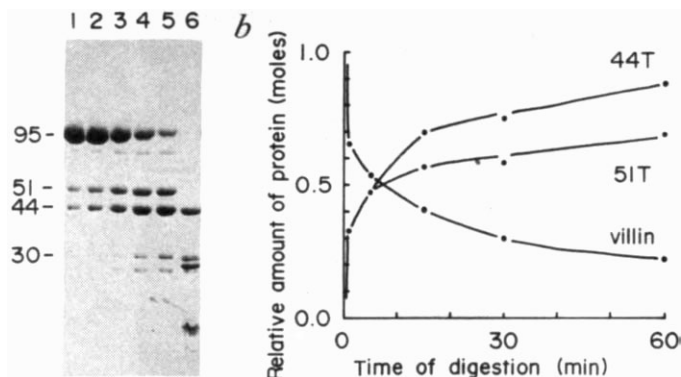


Fig. 1 Trypsin cleavage of villin. **a**, Trypsin cleaves villin, M_r 95,000, into two major peptides of M_r 44,000 and 51,000. Lanes 1-6, digestion after 1, 5, 15, 30, 60 and 135 min, respectively. Trypsin, 5 mg ml⁻¹ in 0.1 M Tris pH 7.5, 20 mM CaCl₂, was purified by affinity chromatography to soybean trypsin inhibitor coupled to Sepharose 4B. The column was washed with tryptamine (5 mg ml⁻¹ in the Tris-CaCl₂ buffer) to remove chymotrypsin. Trypsin was eluted from the column with 3 mM HCl, 10 mM CaCl₂ and stored at -20 °C. Villin (5 mg ml⁻¹), purified using the method of Bretscher and Weber³, in digest buffer (50 mM NaCl, 1 mM MgCl₂, 0.1 mM EGTA, 10 mM PIPES pH 7.0), was cleaved by trypsin at room temperature at a 1:5,000 (wt/wt) ratio of trypsin/villin. The digest was quenched with phenylmethylsulphonyl fluoride (PMSF) to 0.1 mM after 1, 5, 15, 30 and 60 min (lanes 1-5). Another aliquot of villin was digested at 1:500 for 135 min (lane 6). The digests were mixed with SDS sample buffer and electrophoresed³¹ on 7-25% microslab gels³². **b**, The amount of the two villin tryptic fragments increases with time at similar rates. The gels of the trypsin digest were measured by densitometry and the peaks cut out and weighed. The weights of the peaks were converted to moles of peptide assuming equal dye binding. The amount of peptide present was plotted as a function of time of digestion.

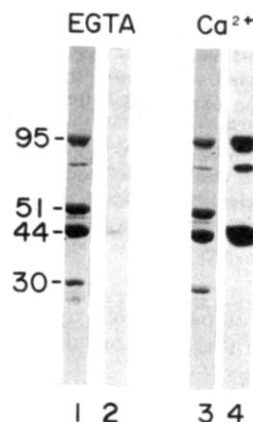
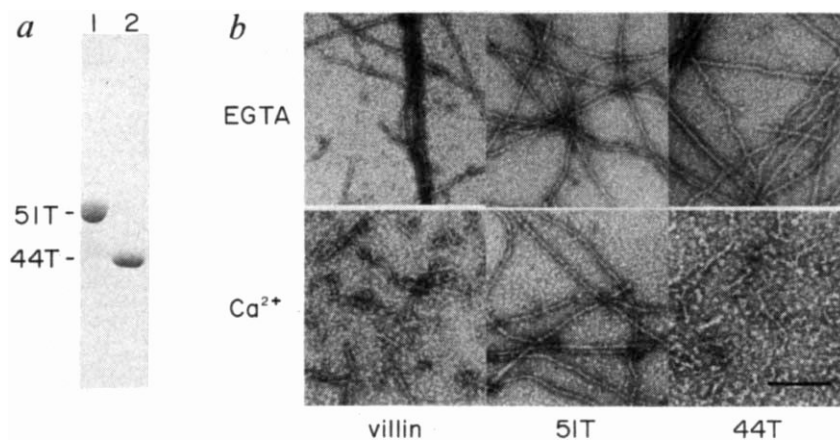


Fig. 2 Identification of calcium-dependent actin-binding fragments by a ¹²⁵I-actin gel overlay assay¹⁹. SDS gels of incomplete villin digests were incubated with ¹²⁵I-actin in 1 mM EGTA (lane 1) or 0.5 mM CaCl₂ (lane 3). Their corresponding autoradiograms (lanes 2 and 4) show only binding in calcium to villin, 44T and an 80,000- M_r contaminant.

Methods. An incomplete tryptic digest of villin was electrophoresed on SDS microslab gels and processed for actin binding. Duplicate samples were electrophoresed on one gel. The gels were fixed in 50% methanol, 10% acetic acid for 15 min, incubated in 10% ethanol for 2 h and then in phosphate-buffered saline (PBS) for 2 h, all at room temperature. After incubating overnight at 4 °C or for 2 h at room temperature with ¹²⁵I-actin (10,000 c.p.m. ml⁻¹, 100,000 c.p.m. total) in either 0.5 mM CaCl₂ or 1 mM EGTA in PBS, the gels were washed, stained, destained and dried. The dried gels were then autoradiographed for 8-36 h at -70 °C in cassettes fitted with Cronex intensifying screens.

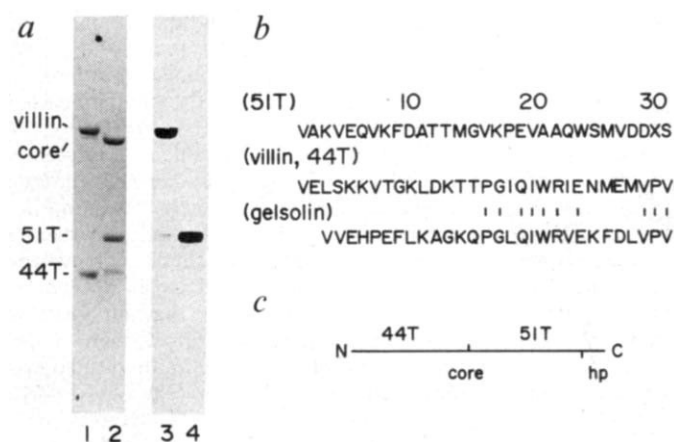
* Present address: Whitehead Institute for Biomedical Research and Department of Biology, MIT, Nine Cambridge Center, Cambridge, Massachusetts 02142, USA.

Fig. 3 Identification of the 44T and 51T actin-binding activities. *a*, The gel of the 51T (lane 1) and 44T (lane 2) fragments after purification over DEAE and Affi-Gel Blue chromatography. *b*, Electron microscopy of F-actin mixed with villin, 44T or 51T fragments. In EGTA (top panel), villin bundles filaments while 44T and 51T have no effect on actin filament organization. In calcium (bottom panel), 44T and villin both sever filaments into short pieces. In these conditions, 51T has no effect on actin filaments. Scale bar, 100 nm.



Methods. *a*, 44T and 51T purification. The villin digest was loaded onto DEAE-Sephacel (10 ml bed volume) previously equilibrated in digest buffer. The bound protein was eluted by a 25–250 mM NaCl gradient. The major peak eluted at 125 mM NaCl and contained 51T. The 51T fractions were made 10 mM with 2-[*N*-morpholino]ethane sulphonic acid (MES), the pH adjusted to 5.0 and loaded onto an Affi-Gel Blue (Bio-Rad, 10 ml bed volume) column equilibrated with 10 mM MES pH 5.0. The column was developed with a 0–0.5 M NaCl gradient in 10 mM MES. The major peak contained only 51T and was concentrated against solid sucrose, dialysed against 50% glycerol, 100 mM NaCl, 0.1 mM EGTA, 10 mM imidazole and then stored at -20°C . 44T does not bind DEAE-Sephacel in digest buffer. The flow-through fraction from the DEAE column was collected and loaded immediately onto Affi-Gel Blue (10 ml bed volume) which had been previously equilibrated with digest buffer. The column was eluted with a 0–1 M NaCl gradient. The major peak which contained 44T was concentrated with sucrose and stored as described above. All steps were performed at 4°C . Protein concentrations were measured using a modified Lowry method³³. The purified fragments were electrophoresed on SDS gels to check for purity. A typical preparation yielded 20 mg of 44T and 7 mg of 51T from 50 mg of villin. *b*, The purified fragments were mixed with rabbit skeletal muscle F-actin ($2\text{ }\mu\text{M}$ final concentration) at a 1:20 molar ratio in 150 mM NaCl, 1 mM MgCl_2 , 10 mM PIPES pH 7.0 and with either 0.1 mM CaCl_2 or 0.1 mM EGTA for 30 min at room temperature. The samples were applied directly to carbon films on 400-mesh grids, rinsed with buffer, negatively stained with 2% uranyl acetate and then examined in the electron microscope. The micrographs were printed to the same final magnification.

Fig. 4 Localization of the 44T and 51T fragments in villin. *a*, Purified villin, 44T (lane 1), villin core and 51T (lane 2) were electrophoresed on 10% microslab gels and then immunoblotted with a rabbit polyclonal antiserum (R200.2) raised against purified villin headpiece (lanes 3, 4). The blot shows that the headpiece antiserum recognizes villin (lane 3), villin headpiece (not shown) and 51T (lane 4), but not villin core (lane 4) or 44T (lane 3). *b*, Amino-acid sequence of the amino termini of villin, 44T and 51T compared with the amino terminus from rabbit macrophage gel-solin. Identical residues are indicated by vertical lines. *c*, Diagram of the locations of the 44T and 51T fragments relative to villin core and headpiece (hp).



Methods. *a*, Villin headpiece was purified from a V-8 digest of villin by a new method. Villin (5 mg ml^{-1} in digest buffer) was cleaved with *Staphylococcus aureus* protease (1:2,000 (w/w) enzyme/protein, 45 min, room temperature) and then applied to a DEAE-Sephacel column previously equilibrated in digest buffer. Villin headpiece does not bind to the column. The flow-through fraction was collected and applied to Affi-Gel Blue equilibrated with digest buffer. The column was eluted with a 0–1 M NaCl gradient in digest buffer. The headpiece-containing peak was pooled and concentrated as above. Villin core was eluted from the DEAE column with a 50–250 mM NaCl gradient in digest buffer. The peak containing villin core was pooled, concentrated against sucrose and then dialysed against 50% glycerol. Villin core was then stored at -20°C . The identity of the headpiece fragment was checked by manually sequencing³⁴ the first four residues from the amino terminus of the fragment. Antisera were raised in rabbits by injecting $80\text{ }\mu\text{g}$ in complete Freund's adjuvant at multiple sites in the back, followed by subsequent injections at 2-week intervals in incomplete Freund's adjuvant. The rabbits were bled out at week 8. The titres of the antisera were quantified by enzyme-linked immunosorbent assay (ELISA). As judged by immunoblots and quantitative ELISA, the preimmune serum contained no detectable cross-reactivity against villin or its proteolytic fragments (not shown). The antiserum was characterized by immunoblotting³⁵ against villin, villin core, 44T and 51T after electrophoresis through microslab gels and electrophoretic transfer to nitrocellulose filters. The filters were blocked with 2% bovine serum albumin, incubated with antiserum at a 1:2,000 dilution and then with swine anti-rabbit IgG coupled to peroxidase (Dako) at a 1:400 dilution. The filters were developed with 4-chloro-1-naphthol. *b*, Purified villin, villin core, 44T and 51T (20 nmol each) were dialysed extensively against 5% formic acid, freeze-dried and subjected to the Edman degradation procedure using a modified Beckman 890B automated sequencer³⁶. The phenylthiohydantoin (PTH) amino acids were analysed and identified by HPLC. The position and identity of each residue were determined at least twice by sequencing different preparations of protein. The villin sequences were checked for homology with protein sequences in the Doolittle data bank³⁷.

actin-binding domain seems to lie within a 44,000-*M*. region of villin. The calcium dependence of binding rules out nonspecific binding as a possible explanation of our results. A minor contaminant (*M*, 80,000) in the preparation also bound actin in a calcium-dependent manner, but its presence varied between experiments and was probably caused by a contaminating protease.

The actin-binding properties of 44T and 51T were then examined more directly by electron microscopy. Fragments were

purified by ion-exchange and Affi-Gel Blue column chromatography. At neutral pH, 51T but not 44T binds to DEAE-Sephacel and thus the fragments can be easily separated. Each fragment separately can then be purified from minor contaminants by chromatography over Affi-Gel Blue columns. The purified fragments (Fig. 3*a*) were mixed with rabbit skeletal muscle actin at a 1:20 molar ratio in conditions^{3–5} in which the intact villin molecule can bundle or fragment actin filaments (Fig. 3*b*). In EGTA, only villin bundled the actin filaments; the 44T and 51T

fragments had no effect on actin filament organization. In calcium, both villin and 44T shortened actin filaments to small pieces while 51T had no effect on actin filament integrity. Thus, electron microscopy directly confirms that the gel overlay assay has correctly identified 44T as a calcium-regulated actin-binding fragment of villin. Although we have not used more quantitative methods^{15,21,22} to test whether 44T caps, nucleates and severs actin filaments in an identical manner to villin, the electron microscopic results suggest that severing can occur.

We surmised that 44T and 51T represent different regions of villin from the kinetics of their appearance during proteolysis. We then used antisera and protein sequence information to identify the location of these fragments more precisely within the villin molecule. We raised a polyclonal antiserum against the villin headpiece, which retains the carboxy terminus of villin^{13,14}. Immunoblots of the villin proteolytic fragments showed that the antiserum recognized villin and 51T but not villin core or 44T (Fig. 4a), suggesting that 51T is associated with the carboxy-terminal half of villin while 44T could originate from the amino-terminal half. This was confirmed by sequencing the amino termini of villin, 51T and 44T (Fig. 4b). The first 30 residues in their sequences showed that villin and 44T share the same amino terminus and, therefore, 44T comprises the amino-terminal half of villin.

In addition, comparison of the villin amino-terminal sequence with the published amino-terminal sequence of rabbit macrophage gelsolin⁶ revealed a short stretch (residues 16–31) of homology. This region shares 10 identical residues and 6 conserved replacements (Fig. 3b). Of these six replacements, five are consistent with a single base change. Although previous studies^{1,9,20,23} have stressed that villin and gelsolin have different locations in cells, produce different peptide maps and elicit antisera which do not cross-react, our sequence information provides structural evidence that explains the similar actin-severing activities of these two proteins.

Our results (summarized in Fig. 4c) document a calcium-regulated actin-binding region which lies within the amino-terminal 44,000-*M_r* fragment of villin. As seen by electron microscopy, this fragment, like villin, villin core and gelsolin, seems to sever actin filaments into short pieces. The similarity between villin and gelsolin in their actin-severing activity as well as in the sequences of their amino termini suggests that villin could be a member of a related family of actin-severing proteins. This family could also include other proteins having *M_s* and calcium-regulated actin-binding activities similar to the villin 44T domain, for example, severin from amoeba^{24,25}, fragmin and cap proteins from slime moulds^{26–29}, and an actin-binding protein from sea urchin eggs³⁰. We speculate that these proteins could have sequence homology in regions corresponding to their actin- and calcium-binding sites.

We thank Drs A. Weeds, J. Kendrick-Jones and H. Huxley for their guidance and support, Mr B. Pope for instructing us on the gel overlay assay, and Dr C. Schutt for stimulating our interest in villin domains. P.M. was supported by an EMBO Long Term Fellowship and a British-American Heart Fellowship.

19. Snabes, M. C., Boyd, A. E. & Bryan, J. *J. Cell Biol.* **90**, 809–812 (1981).
20. Snabes, M. C., Boyd, A. E. & Bryan, J. *Expl. Cell Res.* **146**, 63–70 (1983).
21. Bonder, E. M. & Mooseker, M. S. *J. Cell Biol.* **96**, 1097–1107 (1983).
22. Walsh, T. D., Weber, A., Higgins, J., Bonder, E. M. & Mooseker, M. S. *Biochemistry* **23**, 2613–2621 (1984).
23. Markey, F., Persson, T. & Lindberg, U. *Biochim. biophys. Acta* **709**, 122–133 (1982).
24. Brown, S. S., Yamamoto, K. & Spudich, J. *J. Cell Biol.* **93**, 205–210 (1982).
25. Yamamoto, K., Pardee, J. D., Reidler, J., Stryer, L. & Spudich, J. A. *J. Cell Biol.* **95**, 711–719 (1982).
26. Hasegawa, T., Takahashi, S., Hayashi, H. & Hatano, S. *Biochemistry* **19**, 2677–2783 (1980).
27. Hinssen, H. *Eur. J. Cell Biol.* **23**, 225–233 (1981).
28. Hinssen, H. *Eur. J. Cell Biol.* **23**, 234–240 (1981).
29. Maruta, H., Knoerzer, W., Hinssen, H. & Isenberg, G. *Nature* **312**, 424–427 (1984).
30. Wang, L. & Spudich, J. A. *J. Cell Biol.* **99**, 844–851 (1983).
31. Laemmli, U. K. *Nature* **227**, 680–685 (1970).
32. Matsudaira, P. T. & Burgess, D. R. *Analyt. Biochem.* **87**, 386–396 (1978).
33. Schacterle, G. & Pollack, R. *Analyt. Biochem.* **51**, 654–655 (1973).
34. Chang, J. Y., Brauer, D. & Wittman-Liebold, B. *FEBS Lett.* **93**, 205–214 (1978).
35. Towbin, H., Staehelin, T. & Gordon, J. *Proc. natn. Acad. Sci. U.S.A.* **76**, 4350–4354 (1979).
36. Runswick, M. R. & Walker, J. E. *J. biol. Chem.* **258**, 3081–3085 (1983).
37. Doolittle, R. F. *Science* **214**, 149–159 (1981).

Local protein–DNA interactions may determine nucleosome positions on yeast plasmids

Fritz Thoma & Robert T. Simpson

Laboratory of Cellular and Developmental Biology, Bldg 6, Rm B1-26, National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20205, USA

The structure of the nucleosome core particle, the basic structural subunit of chromatin, is well known¹. Although nucleosomes often appear to be positioned randomly with respect to DNA sequences, in some cases they seem to occupy precisely defined positions on the DNA^{2–8}. The yeast plasmid TRP1ARS1 contains three precisely positioned, stable nucleosomes, I, II and III, which are flanked by nuclease-sensitive regions.⁵ Our aim in the present study was to determine whether the positions of these three nucleosomes relate to (1) protein–DNA interactions; (2) the limited space between nuclease-sensitive regions, which is just long enough to accommodate three yeast nucleosomes (that is, boundary conditions⁹); or (3) proximity to the putative origin of replication in one of the nuclease-sensitive regions^{5,10}. We have tested these alternatives by analysing the positions of nucleosomes after insertion of various lengths of DNA into this region and assembly of chromatin *in vivo*. Our results suggest that specific protein–DNA interactions are the most likely determinants of these nucleosome positions.

We chose as the DNA to be inserted segments of a sea urchin 5S sequence which forms a positioned nucleosome *in vitro*¹¹. An appropriate site at which to insert DNA fragments lies between the strong micrococcal nuclease cutting site in chromatin at position 1,022 and the strongly protected cutting site at 1,082 (Fig. 1). A shift of nucleosome I would prevent cutting at 1,022 and/or would expose the DNA cutting site at position 913. A shift of nucleosomes II and III would expose the cutting site at 1,341 or even that at 1,082. To obtain a suitable cloning site, we introduced a 10-base pair (bp) *Bam*HI linker into the *Nae*I site at position 1,069. Insertions of 75, 155 or 300 bp derived from the 5S gene were made (see Fig. 2). The plasmids derived in this way were transformed into yeast for assembly into chromatin. All constructs gave similar transformation efficiencies and yields in plasmid chromatin preparations; the inserts do not grossly interfere with either *TRP1* or *ARS1* function.

The plasmids were partially purified⁵ and their structures analysed by micrococcal nuclease digestion. All plasmids had DNA packed in nucleosomes (not shown). To determine the locations of the nucleosomes, cutting sites were mapped using the indirect end-labelling method^{4–6}. Mapping of the micrococcal nuclease cutting sites within the *TRP1* gene region showed

Received 18 December 1984; accepted 28 February 1985.

1. Bretscher, A. & Weber, K. *Proc. natn. Acad. Sci. U.S.A.* **76**, 2321–2325 (1979).
2. Matsudaira, P. T. & Burgess, D. R. *J. Cell Biol.* **83**, 667–673 (1979).
3. Bretscher, A. & Weber, K. *Cell* **20**, 839–847 (1980).
4. Matsudaira, P. T. & Burgess, D. R. *J. Cell Biol.* **92**, 648–656 (1982).
5. Mooseker, M. S., Graves, T. A., Wharton, K. A., Falco, N. & Howe, C. L. *J. Cell Biol.* **87**, 809–822 (1980).
6. Yin, H. L., Kwiatkowski, D. J., Mole, J. E. & Cole, F. S. *J. biol. Chem.* **259**, 5271–5276 (1984).
7. Yin, H. L. & Stossel, T. P. *Nature* **281**, 583–586 (1979).
8. Wang, L. & Bryan, J. *Cell* **25**, 637–649 (1981).
9. Yin, H. L., Albrecht, J. H. & Fattoum, A. *J. Cell Biol.* **91**, 901–906 (1981).
10. Harris, H. E. & Weeds, A. G. *Biochemistry* **22**, 2728–2741 (1983).
11. Harris, D. A. & Schwartz, J. H. *Proc. natn. Acad. Sci. U.S.A.* **78**, 6798–6802 (1981).
12. Thorstenson, R., Utter, G. & Norberg, R. *Eur. J. Biochem.* **126**, 11–16 (1982).
13. Glenney, J. R. & Weber, K. *Proc. natn. Acad. Sci. U.S.A.* **78**, 2810–2814 (1981).
14. Glenney, J. R., Geisler, N., Kaulfus, P. & Weber, K. *J. biol. Chem.* **256**, 8156–8161 (1981).
15. Glenney, J. R., Kaulfus, P. & Weber, K. *Cell* **24**, 471–480 (1981).
16. Hesterberg, L. K. & Weber, K. *J. biol. Chem.* **258**, 359–364 (1983).
17. Hesterberg, L. K. & Weber, K. *J. biol. Chem.* **258**, 365–369 (1983).
18. Werke, V. & Weber, K. *Eur. J. Cell Biol.* **31**, 249–255 (1983).

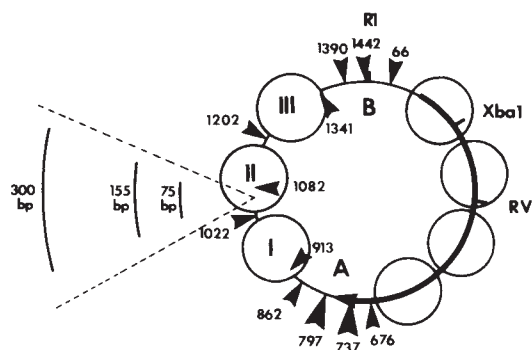


Fig. 1 Chromatin structure of the TRP1ARS1 plasmid: location of the inserts used to study nucleosome positioning. The detailed structure of TRP1ARS1 chromatin is shown⁵, with the open reading frame for the *TRP1* gene indicated by the heavy arrow. Two nucleosome-containing regions are separated by two nuclease-sensitive regions A and B. A is located at the 3' end of the *TRP1* gene and the putative origin of replication. B is located 5' to the gene. The four nucleosomes on the *TRP1* are imprecisely positioned or unstable. Three stable nucleosomes (I, II and III) are precisely positioned on a sequence with unknown function (UNF). The cutting sites for micrococcal nuclease which are protected in TRP1ARS1 chromatin (913, 1,082 and 1,341) are shown inside the circle; cutting sites that are exposed in chromatin (676, 737, 797, 862, 1,022, 1,202, 1,390, 1,442 and 66) are shown on the outside. DNA fragments of 75, 155 or 300 bp were inserted between positions 1,069 and 1,070, as indicated. Nucleosomes I, II and III are characterized by protection of cutting sites at 913 and 1,082 and partial protection of the site at 1,341. Restriction enzymes used and their cutting sites are: *EcoRI*/1 (RI), *XbaI*/186 (XbaI), *EcoRV*/385 (RV).

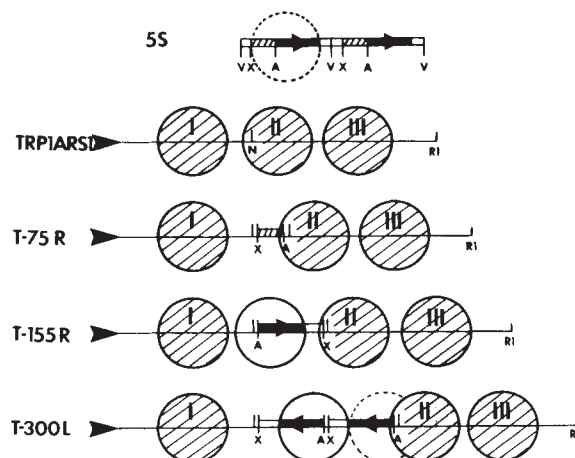


Fig. 2 5S rRNA gene region insertions in the TRP1ARS1 plasmid and nucleosome positions after reassembly in yeast. 5S: Two (of 18) tandemly repeated 190-bp fragments containing 5S sequences are shown. The position of the nucleosome assembled *in vitro*¹¹ is indicated by a dashed circle (from nucleotide 20 to 165 of the published sequence). TRP1ARS1: The positions of the three stable nucleosomes (I, II, III) on the TRP1ARS1 UNF region are shown as hatched circles. T-75R: Using two 10-bp *BamHI* linkers, the 55 bp-long *XmnI*/*AluI* fragment of the 5S DNA (hatched box) was inserted in the same orientation as the *TRP1* gene. T-155R: Using two 10-bp *BamHI* linkers, the 135 bp-long *AluI*/*XmnI* fragment of 5S DNA was inserted in the same orientation as the *TRP1* gene. T-300L: Using three *BamHI* linkers, two 135 bp-long *AluI*/*XmnI* fragments of 5S DNA were integrated in tandem in the opposite orientation to the *TRP1* gene. The *BamHI* linkers are shown as vertical ticks. Restriction sites are: V, *AvaI*; X, *XmnI*; A, *AluI*; RI, *EcoRI*; and N, *NaeI*. The large arrowhead on the left indicates the end of the *TRP1* gene.

Methods. The 1,453 bp-long TRP1ARS1 *EcoRI* fragment was inserted into the *EcoRI* site of a pBR322 derivative, from which the *BamHI* site and all *NaeI* sites were deleted. A *BamHI* linker was inserted into the *NaeI* site of the TRP1ARS1 sequence between nucleotides 1,069 and 1,070 to give plasmid pBRAT2. The insertions in the *BamHI* site were derived from a segment of *Lytechinus variegatus* DNA containing the 5S rRNA gene¹¹. A plasmid was constructed containing 18 tandemly repeated copies of a segment consisting of the DNA from nucleotide 1 to 178 of the published sequence¹¹, flanked by *EcoRI* and *AvaI* sites; the overall repeat length of the insert is 190 bp. The 55 bp-long *XmnI*/*AluI* fragment and the 135 bp-long *AluI*/*XmnI* fragment were gel-purified, attached to *BamHI* linkers and inserted into pBRAT2. The modified TRP1ARS1 *EcoRI* fragments were gel-purified, circularized and used to transform yeast SC-3 (*mata ura3 his3 gal2 gal10 trp1*) obtained from L. W. Bergman (University of Maryland).

that all constructs had unstable nucleosomes similar to those of unmodified TRP1ARS1 (results not shown; see ref. 5). Figure 3 shows the mapping of the micrococcal nuclease cutting sites on chromatin and DNA, from the *EcoRV* site (position 385) counterclockwise using a 199-bp *XbaI*/*EcoRV* fragment as a probe. All the plasmids are strongly cut near the 3' end of *TRP1*, around the putative origin of replication (arrowhead). Further, chromatin from all the plasmids is frequently cut between positions 1,341 and 66. We conclude that insertion of DNA affected neither the chromatin structure of *TRP1* nor the two nuclease-sensitive regions.

In chromatin incorporating plasmid T-75R, strong cutting was observed around position 1,022, accompanied by strong cutting in the inserted DNA region (open bracket in Fig. 3). Cutting sites in this region are weak in naked DNA controls and therefore exposed in chromatin. In the regions of chromatin from position 862 to 1,022, from the DNA insert to 1,202 and from 1,202 to 1,390, prominent naked DNA cutting sites (around 913, 1,082 and 1,341) are protected in a manner similar to that in TRP1ARS1 chromatin. We conclude that nucleosomes are formed at the same positions in the modified plasmid as in TRP1ARS1. The inserted DNA appears to be present as an exposed, long linker between two positioned nucleosomes, I and II (Fig. 2).

In T-155R chromatin, the nucleosomes at positions 862 to 1,022 and 1,202 to 1,390 again occupy identical locations in the derivative and parent plasmids (Fig. 3). Cutting of naked DNA at position 1,082 and at two positions in the insert is strong (relative to cutting at 1,202 and 1,022) while cutting at these three sites in chromatin is weak. As there are ~335 bp between cutting sites 1,022 and 1,202 (including the 155-bp insert), we suggest that there are two tightly packed nucleosomes positioned in this region (Fig. 2).

In T-300L chromatin, protection against micrococcal nuclease cutting between position 862 and 1,022, from the insert to 1,202, and from 1,202 to 1,390 indicates that nucleosomes I, II and III originally resident on the yeast sequences are positioned on

the same sequences. The inserted DNA, however, appears to be cut through a nuclease-sensitive region of ~50 bp at one end and 90 bp at the other end. Strong cutting sites present on naked DNA in the insert are protected in chromatin, suggesting the presence of a single nucleosome on the 300-bp insert. To localize precisely this nucleosome on the inserted DNA, we have mapped micrococcal nuclease cutting sites clockwise from the *XbaI* site and counterclockwise from the *EcoRV* site at three levels of digestion. Cleavage sites at the boundaries of the linker DNAs are at positions $1,032 \pm 15$ and $1,122 \pm 12$ on one side and $1,266 \pm 9$ and $1,315 \pm 15$ on the other side (note that positions here refer to sites in T-300L, not TRP1ARS1). The protected region covers 144 bp between positions 1,122 and 1,266. With respect to the sea urchin 5S sequence, the nucleosome provides protection from nucleoside 162 on one repeat to 163 on the other (Fig. 2); the additional nucleosome assembled *in vivo* occupies a position on the 5S sequence which is similar to the position obtained in the *in vitro* reconstitution experiments¹¹.

The ARS function in the TRP1ARS1 fragment has been localized between positions 615 and 1,453 (ref. 10). We have

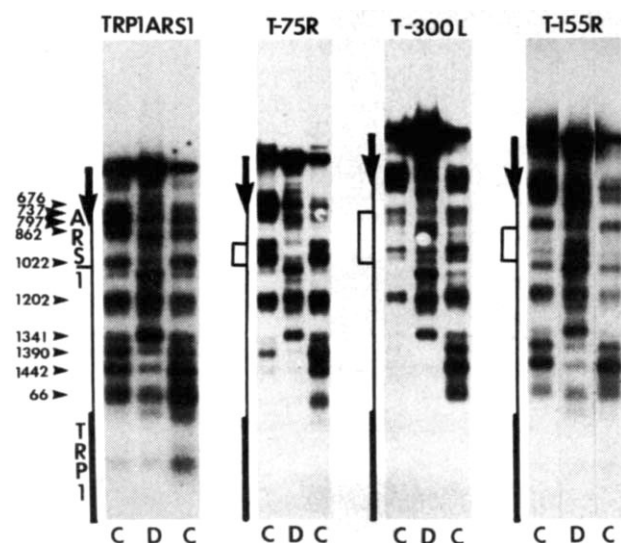


Fig. 3 Mapping of the micrococcal nuclease cutting sites on chromatin (C) and DNA (D) counter-clockwise from the *EcoRV* site. TRP1ARS1: Mapping data and the mean cutting sites, indicated on the left-hand side, are taken from Thoma *et al.*⁵, and serve as a standard. The position of the insertions is marked by a tick. T-75R, T-300L, T-155R: The positions of the inserted sequences are indicated by open boxes. The vertical arrow indicates the open reading frame of *TRP1*.

Methods. Preparation of plasmid chromatin and control DNA, digestion with micrococcal nuclease and mapping of the cutting sites were done in a similar way to that described by Thoma *et al.*⁵. Yeast cells were grown at 30 °C in minimal medium supplemented with uracil and histidine (0.67% yeast nitrogen base without amino acids (Difco), 2% dextrose, 20 g uracil per l, 20 g histidine per l) to an absorbance of 1–4 at 600 nm, washed twice with water and converted to spheroplasts using Zymolyase 60,000 (Kirin Breweries, Takasaki, Japan). The spheroplasts were washed in yeast wash buffer (40 mM K-phosphate pH 7.5, 1 M sorbitol, 1 mM phenylmethylsulphonyl fluoride (PMSF)) and lysed in buffer A (20 mM Tris-HCl pH 8, 150 mM NaCl, 5 mM KCl, 1 mM EDTA, 1 mM PMSF) containing 0.2% Triton X-100. The genomic chromatin was pelleted at 27,000 g at 0 °C for 30 min and the small contaminating material was removed by gel chromatography on Sephacryl S300 in buffer A. The plasmid chromatin eluted in the void volume and was pooled. DNA from one half of the chromatin pool was extracted using phenol, phenol/chloroform, and chloroform; RNA was digested with RNase A, and DNA was re-extracted as above and precipitated twice. Micrococcal nuclease digestions were done in buffer A supplemented with 5 mM CaCl₂ for 5 min at 37 °C using four different enzyme concentrations. To map the micrococcal nuclease cutting sites, DNA was extracted as above, cut with *EcoRV*, separated on 1% agarose gels in Tris-borate buffer containing ethidium bromide, blotted onto nitrocellulose, and hybridized to radioactively labelled *XbaI*/*EcoRV* fragments. The radioactive bands were detected using Kodak XAR-5 film.

shown that different fragments of DNA can be inserted at position 1,069 without disrupting *ARS* function. As the nucleosome between 862 and 1,022 in TRP1ARS1 is highly stable⁵, we suggest that the functional *ARS* sequence lies in the nuclease-sensitive region A between 676 and 862, in analogy to simian virus 40, where the origin of replication is known to be in a nuclease-sensitive region¹².

We next consider the possible determinants of nucleosome positions. Insertion of 75–300 bp of DNA had no effect on the positions of nucleosomes I, II and III. Surprisingly, yeast prefers to assemble long linkers (75, 49, 90 bp), although on average the yeast micrococcal nuclease repeat length is ~160 bp¹³, consistent with 145-bp nucleosome cores connected by ~15-bp core-to-core linkers.

One may ask whether nucleosome formation near the replication fork aligns or phases nucleosomes in positions which are

determined by the location of the origin of replication. Although the precise location of the origin of replication and the nature of the replication process are unknown, the existence of variable-length linkers in T-75R and T-300L excludes the possibility that nucleosome positioning is a simple function of replication.

The existence of long linker DNA demonstrates that the yeast did not take the opportunity to rearrange or randomize its nucleosome positions; this observation excludes a simple boundary mechanism in which the positions of nucleosomes are determined by a statistical arrangement within limited space between two boundaries, regions which interact with other proteins to preclude nucleosome formation⁹. Although we must consider the possibility that the inserted DNA itself (particularly the DNA which exists as long linkers) may represent a boundary, this seems unlikely for the linkers in T-75R and T-300L. Thus, these sequences can associate with histones to form nucleosomes, that is, the X-A fragment in T-75R formed *in vitro*¹¹ and the sequences represented by both the open and black boxes in T-300L in the additional nucleosome formed *in vivo* (Fig. 2). As we have excluded the simple boundary mechanism and a replication-related mechanism, we conclude that local protein-DNA interactions, involving either histones *per se* or positioning proteins¹⁴, probably determine the positions of these yeast nucleosomes.

The nucleosome formed on the 300-bp insert appears to occupy a position identical to that of the core particle assembled *in vitro*¹¹. As only 90 bp of sequence are shared in the nucleosomes assembled *in vitro* and *in vivo*, we suggest that this sequence (*AluI* site to position 162) must contain a nucleosome positioning signal. A similar conclusion has been reached in studies of the *in vitro* assembly of modified 5S fragments with histones, although *in vitro*, sequences to the left of the *AluI* site serve a discriminatory function in phasing (P. FitzGerald and R.T.S., unpublished observations). As only histone-DNA interactions were necessary *in vitro* to position the nucleosome, we infer that, in yeast, histone-DNA interactions may be the dominant mechanism for positioning of this particular nucleosome *in vivo*. It is interesting that chicken erythrocyte histones were used for the *in vitro* experiments¹¹, therefore the positioning signal may be universal.

Whatever mechanisms are responsible for positioning of these nucleosomes, the fact that only one of the two positioning signals on the 5S DNA was used in T-300L (formation of the nucleosome indicated by the dashed circle in Fig. 2 did not occur), together with the observation that nucleosome II did not shift in T-75R or T-300L, suggests that a hierarchical array of positioning signals must exist. The signal for nucleosome II must be stronger than that for the 5S sequence.

We have shown that yeast and yeast plasmids can be used as an *in vivo* chromatin reassembly system for addressing questions relating to structure and function of nucleosomes *in vivo*. Although the present experiments have analysed structural changes only in circular plasmids, homologous recombination in yeast will allow the extension of such studies to the chromosome itself.

We thank our colleagues for criticisms and discussion, particularly David Pederson and Peter FitzGerald for comments on the manuscript and Joel Brubaker for plasmid construction and isolation.

Received 4 December 1984; accepted 19 February 1985.

1. Richmond, T. J., Finch, J. T., Rushton, B., Rhodes, D. & Klug, A. *Nature* **311**, 532–537 (1984).
2. Igo-Kemenes, T., Hoerz, W. & Zachau, H. A. *Rev. Biochem.* **51**, 89–121 (1982).
3. Bloom, K. S. & Carbon, J. *Cell* **29**, 305–317 (1982).
4. Wu, C. *Nature* **286**, 854–860 (1980).
5. Thoma, F., Bergman, L. W. & Simpson, R. T. *J. molec. Biol.* **177**, 715–733 (1984).
6. Nedospasov, S. A. & Georgiev, G. P. *Biochem. biophys. Res. Commun.* **92**, 532–539 (1980).
7. Cartwright, I. L. *et al. CRC crit. Rev. Biochem.* **13**, 1–86 (1982).
8. Varshavsky, A. J., Sundin, O. H. & Bohn, M. J. *Cell* **16**, 453–466 (1978).
9. Kornberg, R. *Nature* **292**, 579–580 (1981).
10. Tschumper, G. & Carbon, J. *Gene* **10**, 157–166 (1980).
11. Simpson, R. T. & Stafford, D. W. *Proc. natn. Acad. Sci. U.S.A.* **80**, 51–55 (1983).
12. Varshavsky, A. J., Sundin, O. H. & Bohn, M. J. *Nucleic Acids Res.* **5**, 3469–3478 (1978).
13. Lohr, D., Kovacic, R. T. & Van Holde, K. E. *Biochemistry* **16**, 463–471 (1977).
14. Strauss, F. & Varshavsky, A. *Cell* **37**, 889–901 (1984).

Determination of surface topography of biological specimens at high resolution by scanning tunnelling microscopy

A. M. Baró*, R. Miranda*, J. Alamán*, N. García*,
G. Binnig†, H. Rohrer†, Ch. Gerber†
& J. L. Carrascosa‡

* Departamento de Física Fundamental, C-III, and

‡ Centro de Biología Molecular (CSIC-UAM),

Universidad Autónoma de Madrid, Cantoblanco, 28049-Madrid, Spain

† IBM Zurich Research Laboratory, 8803-Rüschlikon, Switzerland

Although techniques are available for the determination of the three-dimensional structure of biological specimens, for example scanning electron microscopy, they all have some serious drawback, such as low resolution, the requirement for crystals or for the sample to be analysed in a high vacuum. In an attempt to develop a technique for high-resolution three-dimensional structure analysis of non-crystalline biological material, we have tested the applicability of scanning tunnelling microscopy (STM), a method that has been used successfully in the analysis of metal and semiconductor surface structures¹⁻³. We report here that scanning tunnelling electron microscopy can be used to determine the surface topography of biological specimens at atmospheric pressure and room temperature, giving a vertical resolution of the order of 1 Å. Our results show that quantum mechanical tunnelling of electrons through biological material is possible provided that the specimen is deposited on a conducting surface.

We have used a STM microscope similar to the one developed at the IBM Zürich Laboratory¹, but operated at air atmospheric pressure. The microscope works by scanning a metal tip of very small radius at a distance ~ 5 – 10 Å above the surface by means of three mutually perpendicular piezoelectric ceramics. In the constant-current mode, the tip-surface distance remains roughly constant during the scanning because of the extreme sensitivity of the tunnel current with respect to the tip-surface separation⁴.

To proceed with the STM of biological samples, a flat conducting and structureless substrate was needed. This condition was satisfied by pyrolytic graphite (Fig. 1a), where a surface which is atomically flat on a $\sim 2,000$ -Å range displays a step of monoatomic height. From this image, we could estimate the stability and resolution of our microscope to be ~ 1 Å of vertical resolution and ~ 5 – 10 Å of lateral resolution. These values were obtained at atmospheric pressure and room temperature.

We have analysed two types of biological samples, both derived from bacteriophage $\phi 29$ particles. This small bacteriophage is composed of a prolate icosahedral head (415×315 Å) and a complex tail (410 Å long)^{5,6}. Phage particles release their DNA and part of their neck-tail complexes when treated with EDTA⁶. The purified phage samples were diluted in 0.1% glycerol for examination by STM. A control experiment to check the influence of the 0.1% glycerol solution on the graphite surface demonstrates a noise level of the surface lines only slightly higher than that of the graphite substrate (Fig. 1b).

The phage-derived structures were allowed to air dry over an electron microscope grid and then stained to detect the degree of destruction provided by this drastic drying procedure. Figure 2a shows that phage particles (either with or without DNA) retained their characteristic gross morphology^{5,6}. A parallel sample was then adhered to a surface of freshly cleaved graphite by drop deposition, allowed to air dry and then examined by STM.

Over a flat background (similar to that shown in Fig. 1b), a series of reproducible scans allow us to define objects (Fig. 2b–d). A comparison of these surface topographies with the electron micrographs of the phage-derived structures suggested that the STM topographic profiles could be correlated qualitatively with the general shape and dimensions of DNA-filled phage, empty head and empty phage particles. Flattening of the

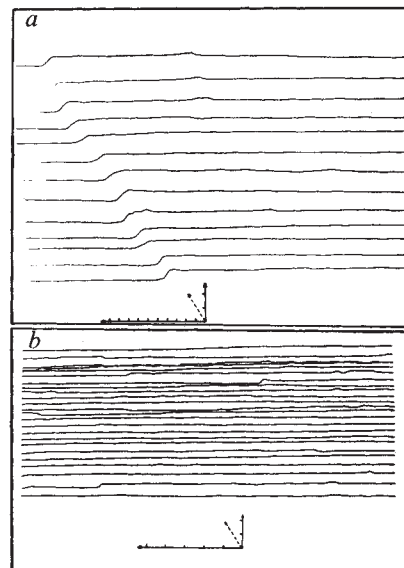


Fig. 1 a, STM topographic curves of a pyrolytic graphite surface cleaved in air, obtained at ambient pressure. Divisions on the axes are 10 Å apart. The scale along the y-axis (broken line) is not well determined. A step of height 7 Å corresponding to the lattice constant of graphite (6.708 Å) is clearly visible. b, STM topographic curves of a graphite surface covered with 0.2 M ammonium bicarbonate containing 0.1% glycerol used to deposit the bacteriophage-derived structures. Divisions on the axes are 50 Å. Note that the substrate is flat over the range $\sim 1,000$ Å.

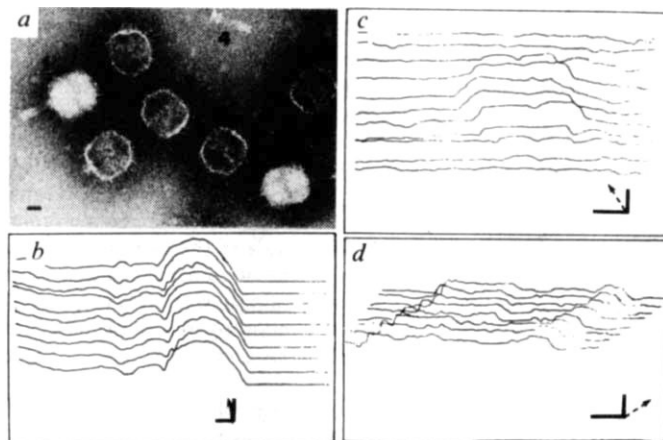


Fig. 2 a, Electron micrograph of various structures derived from bacteriophage $\phi 29$. Phage particles treated with EDTA⁶ released their DNA and part of their neck-tail complexes. After partial purification of the empty head fraction, the material was diluted 10 times in 0.1% glycerol and allowed to air dry over a collodion-carbon-coated electron microscope grid. After drying, the sample was negatively stained with 2% uranyl acetate and observed by transmission electron microscopy. Numbers 1, 2 and 3 correspond, respectively, to DNA-filled phage particle, empty head and empty head with tail. Neck-tail complexes (number 4) can also be seen. Scale bars, 100 Å. b–d, STM surface profiles from a parallel sample dried over graphite. Profiles in b probably correspond to scans along the head-tail axis of a DNA-containing phage particle. Profiles in c can be correlated with the expected flattened structure obtained after air drying an empty capsid without tail. Profiles in d can be interpreted as scans along the head-tail axis of an empty phage particle with tail complex. Note that the height of the flattened head in c and d (~ 70 Å) is much smaller than in the case of the DNA-containing head shown in b (~ 200 Å) whereas the height of the tails in b and d is very similar (~ 75 Å).

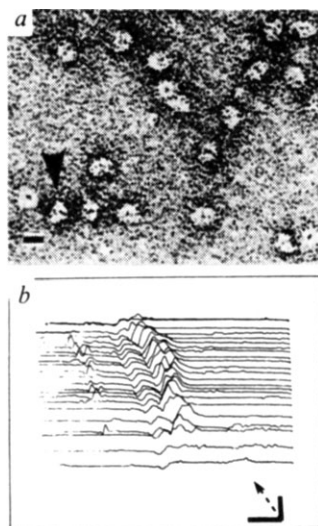


Fig. 3 *a*, Electron micrographs of the upper collar of bacteriophage $\phi 29$. The upper collar protein of phage $\phi 29$ was purified^{8,9} and dialysed against 0.2 M ammonium bicarbonate containing 0.1% glycerol to prevent destruction during the air-drying procedure. This sample was negatively stained with 2% uranyl acetate and observed by transmission electron microscopy. The collars appear as circular structures with a hole in the centre. Occasional side views show a dumbbell shape with a hole running through the centre of the neck (arrowhead). Scale bar, 100 Å. *b*, STM surface topography curves obtained from a collar-containing sample deposited over graphite and air-dried. The scans show profiles of a structure (100 Å in diameter along the *x*-axis and 80–90 Å in height along the *z*-axis) that correlates with the collar structure inferred from the images shown in *a* (ref. 9).

air-dried specimens, commonly observed in all electron microscope methods⁷, is also seen in the present experiments for empty viral heads (Fig. 2*c, d*). Repeated scans over the same area always showed very similar topography, implying that STM does not drastically alter the biological surface structure.

The second sample studied by STM was the oligomeric protein structure that forms the upper collar of $\phi 29$ (refs 8, 9). Transmission electron micrographs of this oligomer showed a circular front view (135 Å diameter) with a hole in the centre (Fig. 3*a*). Occasional side views showed that the oligomer has a dumbbell shape (125 Å on height). The collar sample was dialysed against 0.2 M ammonium bicarbonate (containing 0.1% glycerol) to remove salts and low-relative molecular mass components and then analysed by STM.

Over a general flat surface, some series of scans were observed that could be correlated with the presence of the phage collars (Fig. 3*b*): the more characteristic feature is a structure ~100 Å in diameter (*x*-axis) and 80–90 Å in height (*z*-axis) showing a hole in the centre, which is in good agreement with the structure of this collar observed by transmission electron microscopy⁹ (Fig. 3*a*).

Most of the pictures presented were scanned insufficiently in the *y*-direction, which prevents the observation of the entire object. This limitation resulted from the difficulty in controlling the piezo movement in the slow direction (*y*-axis), because both piezo relaxation and external scanning may have a similar value.

We stress that we have obtained real corrugated structures in the STM of as much as 250 Å for an applied voltage of 0.4–0.8 eV, giving a tunnel current of 10 nA. Assuming an average tunnel area of 300 Å², as indicated by the experimental lateral resolution⁴, an effective tunnel resistivity of ~1 Ω cm⁻¹ is obtained. This resistivity is much too small for a single tunnel process over ~200 Å. We propose a transport mechanism to explain the effective conductivity of the biological material analysed by assuming the existence of regions where the electrons tunnel (conduction levels higher than the kinetic energy

of the electrons) and regions where they propagate (conduction levels lower than the kinetic energy of the electrons). If such regions of tunnelling and propagation of electrons exist in the specimen, electrons can easily move by percolating in the propagating regions, whereas resonant tunnelling in the tunnel regions can substantially enhance the conductivity over single tunnel processes. Calculations on one-dimensional disordered systems show an enhancement of the conductivity¹⁰. We believe that both type of regions are provided by the different bonds and radicals existing in the protein of the biological material. More information will be provided in a forthcoming paper.

In conclusion, our results show that STM can be used to determine the surface topography of air-dried untreated biological specimens at atmospheric pressure and room temperature without altering the material. Real-space three-dimensional topographic profiles giving a vertical resolution of the order of 1 Å are obtained, which can be correlated in terms of size and shape with biological objects. In addition, STM can be used to determine the surface microstructure of other materials such as metals, semiconductors, thin films and polymers. The operation of the microscope at atmospheric pressure opens the possibility for investigation of the technological applications of these materials in such fields as metallurgy, microelectronics and catalysis.

We thank Professors N. Cabrera, C. López and S. Vieira for their support and Drs G. Ramírez, J. Jiménez and S. Ferrer for fruitful discussions. We also thank IBM, particularly Dr Herb Budd and V. Martínex for financial support, and the Ramón Areces Foundation and the Fondo de Investigaciones Sanitarias for partial financial support. This work was developed by a joint collaboration with the Centro de Investigación UAM-IBM.

Received 21 November 1984; accepted 12 March 1985.

1. Binnig, G., Rohrer, H., Gerber, Ch. & Weibler, E. *Appl. Phys. Lett.* **40**, 178–180 (1982).
2. Binnig, G. & Rohrer, H. (eds) *Proc. 6th gen. Conf. Eur. Phys. Soc.* (Prague, 1984).
3. Binnig, G., Rohrer, H., Gerber, Ch. & Weibler, E. *Phys. Rev. Lett.* **50**, 120–123 (1983).
4. García, N., Ocal, C. & Flóres, F. *Phys. Rev. Lett.* **50**, 2002–2005 (1983).
5. Anderson, D. L., Hickman, D. D. & Reilly, B. E. *J. Bact.* **91**, 2081–2089 (1966).
6. Carrascosa, J. L., Viñuela, E., García, N. & Santisteban, A. *J. molec. Biol.* **154**, 311–324 (1982).
7. Kellenberger, E., Häner, M. & Wurtz, M. *Ultramicroscopy* **9**, 139–150 (1982).
8. Ibañez, C., García, J. A., Carrascosa, J. L. & Salas, M. *Nucleic Acids Res.* **12**, 2351–2365 (1984).
9. Carrascosa, J. L., Carazo, J. M., Ibañez, C. & Santisteban, A. *Virology* (in the press).
10. Stoll, E. & Schnedier, T. *Solid St. Commun.* **11**, 1327–1329 (1972).

Corrigendum

High-affinity binding site for a specific nuclear protein in the human IgM gene

L. Hennighausen, U. Siebenlist, D. Danner, P. Leder, D. Rawlins, P. Rosenfeld & T. Kelly Jr
Nature **314**, 289–292 (1985)

Lines 8 and 9 of Fig. 3 should read “in the absence (lane 4) or presence (lane 3) of the”.

Erratum

Functional modifications of cytotoxic T-lymphocyte T200 glycoprotein recognized by monoclonal antibodies

L. Lefrançois & M. J. Bevan
Nature **314**, 449–452 (1985)

ON page 450, column 2, line 6 of the first paragraph should read “...precipitated proteins with apparent *M_r*s of 240,000 (240K), 220K...”.

Nuts with everything

John Rivers

Health or Hoax? The Truth about Health Foods and Diets.

By Arnold E. Bender.

Elvendon Press, The Old Surgery, High Street, Goring-on-Thames, Reading, Berkshire, UK: 1985. Pp.184. £8.50.

The Penguin Encyclopaedia of Nutrition.

By John Yudkin.

Viking: 1985. Pp.431. £14.95, \$20.

Diet-Related Diseases: The Modern Epidemic.

By Stephen Seely, David L.J. Freed, Gerald A. Silverstone and Vicky Rippere.

Croom Helm/Avi: 1985. Pp. 272. Hbk £19.95, \$35; pbk £9.95.

THE dilemma of nutrition is not merely caught in these three books; it is writ large, as six authors wrestle with what nutritionists do and do not know, and, more importantly perhaps, with what almost everyone else seems so certain about. Each book has a different aim, and a very different style, but they share a common constituency of the layman, the undergraduate and the professional in search of diversion.

Professor Bender's book, a swingeing, shotgun attack on health foods and their purveyors, will clearly attract the greatest attention (an extract has already appeared in the *Daily Mail*). Although I have some reservations about it, a detailed dissection of health food mythology is long overdue and Bender provides this in a most entertaining style. The message of the book is neatly encapsulated in his opening contention, that there are no such things as health foods, there is merely an industry which sells them. Bender not only shows that the claims made for health food products have little or no scientific basis, he casts doubt on the motives, practices and business standards of the industry, concluding briefly with his own recommendations for a good diet. All this is done with gusto, employing a mixture of sarcasm, wit and his encyclopaedic knowledge of nutrition. But while I finished the book with my appetite whetted it was not satisfied: at times I was left wishing for more, or fuller, answers to some of the issues he raises, hoping that he might return to the attack at greater length after each skirmish; at others, in flat disagreement with some of his scientific assertions.

One issue touched on tantalizingly, but not fully explored, is the nature of the health food movement itself. With apparent relish, Bender quotes the views of a colleague who describes it as compounded of both cranks and swindlers "bent on the commercial exploitation of the frightened and helpless", and certainly he cites enough cases to demonstrate the existence of both. But there is clearly more to be said, as when he remarks that "many of the [health-food] magazines are owned by the same organisations that make and sell the health foods". This is indeed "more than

interesting" because through such magazines manufacturers can freely make claims that even the feeble British regulations would forbid them to publish in advertisements. The implication that the followers of the health food movement are encouraged in their views by a gospel of ardent nonsense, surreptitiously funded by manufacturers, is a worrying one. Here is a can of wholemeal worms that should have been opened wider.

Though manipulation of information may in part be the reason for the rapid growth of the health food industry (turnover has increased by 400% in Britain in the past decade, for example), it is not enough to account fully for its success. Bender briefly mentions other factors, such as the general hypochondriasis about food in our society, and the pervasive Western wish to believe in a Golden Age of dietetic innocence, when, guided by nature, we unerringly chose the diet of untarnished pastoral perfection. It is, I think, a pity that he did not examine more fully the reasons why we are so prone to these obsessions, and particularly the role that his (and my) profession has played in encouraging everyone to believe that eating should carry a government health warning.

This is part of a general weakness in the book. In pointing out the error of the health fooders' ways at such length, Professor Bender necessarily asserts continually that the nutritionist knows best. This may be true more often than not, but sometimes he is unduly soft on scientists and complacent about what they have shown or done. When he states that artificial sweeteners are safe because tests have not shown them to be harmful in human beings, or that "apart from premature babies, extra Vitamin E does not have any value", there is a real danger of confusing particularly the lay reader not only about the nature of scientific proof but about the extent of a professional consensus on these issues. On the other side of the coin he sometimes quotes relatively weak scientific evidence as demonstrating a proven danger of health food products, as, for example, in his listing of toxic effects of high doses of Vitamins C, B₆ and B₁, where the professional view (as

evidenced by the other two books discussed here) would be that there is no firm evidence as to the adverse effects of overdoses of these compounds. And while he pronounces long and loud about even putative cases of vitamin poisoning attributable to the advocacy of health food freaks, he says nothing about the crop of deaths of children in Britain 30 years ago which resulted from professional nutritionists advocating too high a level of supplementation of infant foods with Vitamin D: a compound that, as he says, is more poisonous than cyanide.

These remarks are not intended as a defence of the health food business or to detract from Professor Bender's well-justified attack on it. It is just that I regret what I see as unnecessary certainty in his tone because it lessens the force of his criticisms. It is particularly unfortunate in a book which ends with a chapter giving advice on a healthy diet. For what Professor Bender recommends is the currently fashionable recipe of low fat, low saturated fat, less sugar, less salt and more fibre. The sort of advice, in short, which not so long ago nutritionists were wont to ridicule when health food cranks proposed it.

Such errors are inevitable in a science whose discoveries can be implemented so easily, and which has a public avid to hear of each whisper of a miracle. But that makes it necessary for its practitioners to consider carefully everything they say, particularly when writing for the general public. To some Professor John Yudkin would seem an arch-offender against this precept, an eloquent voice who has put his views on the adverse effects of dietary sucrose to the public over the heads of his colleagues. In fact Yudkin has devoted very little of his working life to attacking sucrose. Far more of it was spent in devising the first undergraduate degree in nutrition, a pioneering attempt to provide in a single course a perspective on the diverse disciplines — from cooking to physiology, economic history to chemistry — of which a nutritionist should be aware. In doing that he also fostered neglected areas of the subject, most notably the social and historical, that have suffered because many scientists (and, in Britain, the Research Councils) see nutrition simply as applied biochemistry.

The Penguin Encyclopaedia of Nutrition is a reflection of this side of Yudkin's activities. It is a large book, but neither exhaustive nor exhausting, and an amalgam of some truly nutritional entries and some plucked from the ancillary disciplines that Yudkin has always argued a would-be nutritionist must master. The entry on health foods jostles one on pH, gout is discussed, so is osmotic pressure, nutrient balance and locust beans. Necessarily much is missed out and it seems that Yudkin has frequently chosen to concentrate upon what interests him. There is a long entry

on sucrose, of course, but much more on the history of nutrition, notably the succinct biographies of many of the founders of nutritional science and the accounts of the food habits and nutritional impact of the Neolithic revolution. The air of relaxed authority emerges in entries such as that on "Food", which he calls "a word universally used and presumably universally understood" but not susceptible of a "sufficient and necessary definition" before going on gently to refute the slick definitions found in dictionaries. This a reference work to browse through and ruminate upon. It will be particularly useful to those who feel impelled to pronounce upon nutritional matters, among whom one might include the authors of the third book reviewed here.

Diet-Related Diseases, a collaborative effort by an epidemiologist, an immunologist, a chemist and a clinical psychologist, is a refreshing book but suffers from three things. The first is the unevenness in style, predictable in a multiple-author volume. The second is the cover: a clichéd photograph of a collection of junk foods that fails to reflect the thrust of the book and suggests that the publishers did not fully appreciate what their authors have tried to do. For in no sense is this book clichéd, nor is it particularly concerned to attack or defend junk food. What the authors do is set out what they see as possible roles for diet in many of the diseases of the affluent society, and to present both conventional and novel (and in parts highly idiosyncratic) analyses of this case. The core is a collection of chapters by Stephen Seely which set out the evidence for a dietary role in arterial diseases and various cancers; on the periphery are chapters by his colleagues on food toxicants, food allergies, diet in mental illness, and diet and ageing.

Seely is the author of a provocative series of papers in *Medical Hypotheses* which have tried to cast new light on the well-trodden field of diet and degenerative diseases chiefly by attempting a more thorough analysis of data on national diets and the prevalence data on diseases. While the evidence is set down in the book with commendable clarity, it is the very clarity which makes apparent the occasional, but important, misconception about the quality of the nutritional, and other, assumptions which underpin the arguments.

Here lies the book's third and most serious fault. For example, Seely's analyses make great use of estimates of per capita availability of foodstuffs in various countries, which he calls "food consumption data". They are not: the two may differ by some hundreds of calories and the discrepancy is a notorious source of error. He asserts axiomatically that "a correlation coefficient of 0.9 denotes a degree of positive correlation when causal connection is probable". He suggests that the longevity of the human species is explained by our low level of energy consumption for our size. This is an arguable contention, but it

is presented in a way that suggests Seely is unaware of Rubner's work of a century ago which showed that human beings live four times longer than a comparative study of energy turnover would suggest, or of Flourens's (1856) "law of longevity" that lifespan of different species is about five times as long as the duration of the growth period, a generalization that does fit the human species.

Such errors might yield to a more thorough training in nutrition and a respect for its historical background such as is to be found in Yudkin's *Encyclopaedia*. But though Seely's theories would be better for greater nutritional sophistication, this failing does not destroy the book. What

particularly recommends itself is not the correctness or otherwise of the authors' specific views, it is an overall air of caution, reasonableness and balance in their tone. Even when the writers are expounding their own pet theories they sound more like judges than prosecutors. I hope they produce more books, because dispassionate commentators will be in demand as nutrition increasingly becomes a public health issue which is seen to require missionary zeal on all sides. □

John Rivers is a Senior Lecturer in the Department of Human Nutrition at the London School of Hygiene and Tropical Medicine, University of London.

Biology at work

Norman Carey

Biotechnology and Genetic Engineering Reviews, Vols 1 and 2.

Executive editor Gordon E. Russell.
Intercept, PO Box 2, Ponteland, Newcastle upon Tyne/Scholium, 265 Great Neck Road, Great Neck, New York: 1983/1984. Each volume pp. 450, £50, \$79.50.

AT THE moment that these volumes arrived on my desk for review I was in conversation with one of my colleagues. "Oh no", he said, "not another one. There's too much being written about biotechnology these days." I suppose the comment could mean either that the topics have already been well covered elsewhere or that the speaker does not have time to read all that appears on the subject. Whether this series really is "too much" depends on the objectives and whether they are achieved.

The term "biotechnology" was coined at the time of the revolution in thinking which was brought about by the arrival of recombinant DNA techniques. To many people it has come to mean the industrial application of recombinant DNA. Indeed, it does to some of the authors of articles in these volumes, as is evidenced by the use of the word (Vol. 1, p.231). Of course, although a new (or at least newly publicized) term, by any definition biotechnology covers a multitude of highly profitable industrial activities which long pre-date the recombinant revolution.

In his preface, the editor refers to this fact. He says that the articles are intended to cover biotechnology "in the widest sense of that word". He implies, however, as does the title of the books, that it is the application of recombinant DNA which is the unifying feature of the articles. If that were the intention, then I feel there would be some value in a series such as this. One is forced to conclude, however, that this cannot be the aim, since many of the contributions scarcely mention *in vitro* recombination. The first article in Vol. 1, for exam-

ple, "Applications of Biocatalysts to Biotechnology" (a few years ago this would have been called "Industrial Applications of Enzymes"), mentions genetics and protein engineering once, in the penultimate paragraph of the conclusions.

Perhaps, then, I have read too much into the title and preface. Maybe the series is intended to be a set of reviews on biotechnology and the title just has a high buzz-word quotient. In that case, the volumes should be judged by the quality of the articles and need for a review of each field. While there are a number of similar review series without a recombinant slant, this kind of duplication has not been a disadvantage in any other field.

Most of the contributions are informative and well written. However, there is an unnecessary degree of superficiality and repetition which a firm editorial hand should have dealt with. For example, three of the articles in Vol. 2 contain accounts of how to do cDNA and genomic cloning which are at once too slight to be of value to anyone who wishes to learn the techniques and too familiar to anyone with a degree in biochemistry. Such descriptions are now in the textbooks and do not belong in a series of "up to the minute in-depth reviews", to quote the preface again.

Librarians in industrial laboratories may subscribe to this series. But I think it likely that they will consider that scientists in their companies already know more about the topics in their own fields than these articles contain, and that they would have no reason to refer to such a wide variety of other subjects. However, libraries serving university departments of biochemistry and microbiology should have it. As general reading, most of the articles would be valuable to advanced and even first degree students; moreover, they do provide an excellent picture of the breadth of application of their disciplines. This series shows biotechnology both as high-quality science and as an important and valuable industrial activity. □

Norman Carey is Director of Research and Development at Celltech Ltd, Slough, Berkshire.

Building for the scientist

Michael Brawne

Design, Construction and Refurbishment of Laboratories.

Edited by R. Lees and A. F. Smith.
*Ellis Horwood/Halsted: 1984. Pp.375.
£37.50, \$79.95.*

LABORATORIES are among the most expensive and least permanent of buildings. Any attempt to increase awareness of the difficulties in designing them must be welcomed, and must be seen as a contribution towards more satisfactory solution of an especially intractable building problem. Economics, of course, are often a decisive factor and it is at the same time especially important to keep the apparently high capital cost in perspective: as W.P. Horswell, one of the contributors to this volume, points out, the capital cost of a science department at a British university, calculated as an annual mortgage payment, is only 14 per cent of the running cost — 86 per cent goes to recurrent costs — and is only 7 per cent if buildings and land alone are counted. This, however, is a relationship that rarely influences the decisions of universities and other institutions funding laboratory buildings.

It was the aim of the editors, two scientists at the Laboratory of the Government Chemist in London, that the work of the 40 contributors should build up into a kind of mosaic to cover a wide range of issues and enable the designer of a new or a refurbished laboratory to avoid the major pitfalls. The foreword, moreover, suggests that the information should be widely relevant in both developed and developing countries.

As might be expected, the 36 chapters which make up the book differ both in intention and quality. Many of them contain useful information, clearly set out; some deal with generalities, without in the short space available going beyond these; and a few do no more than to set out checklists, which may be helpful reminders to those who already know the answers but do little to transmit any knowledge. Few say anything original, but there are some surprises. For example, in his paper on safety, N.H. Pearce of Bristol University suggests that the accepted wisdom on fire escapes, enshrined in legislation and enforced by fire officers, may actually make conditions much worse by trapping smoke rather than allowing it to disperse.

Nor is there necessarily unanimity among the contributors. As in science itself there will always be different views of what is most appropriate in a laboratory building. Not only are the final decisions invariably the upshot of a set of value judgements made by the designer, but laboratories are continually changing to reflect advances in

scientific and technological research and to house the new equipment best suited to that research. Developments of the recent past have resulted in a drift towards opposite ends of the scale; towards very large equipment, as in radioastronomy or nuclear research institutions such as CERN, or towards miniaturization. A common factor, however, is that many controlling, measuring and recording instruments are in some way dependent upon computer technology, yet little of that impact is described in the book. At an elementary level, for example, the use of visual display units has serious implications for lighting but no mention of this is made in the pertinent chapter.

A number of the issues covered — on flexibility and adaptability, on safety, on the choice between conversion of an old building or construction of a new one, on reliability principles or building appraisal in use — have clear application to other building types and are thus of general interest. Some of the more particular discussions also have relevance beyond laboratory design since a number of other

types of building — for the manufacture of electronic components or the processing of food — may well need much the same conditions. Thus, for all its failings, the book should have appeal beyond those principally concerned with laboratories.

It should never be forgotten that a laboratory is not only a place of work but is a kind of home for a large number of people. In the conversations I have had with scientists in both the physical and the life sciences for whom I have designed laboratories, the final and most searching questions were always about "what will it be like to be there?". It is assumed that there will be enough fume cupboards or adequate electrical screening; what is difficult to pin down and capture is that elusive sense of place. The book says nothing about this. Perhaps the editors felt that it was unimportant or outside their mandate; so, unfortunately, have too many others who have been responsible for laboratory buildings. □

Michael Brawne is Professor of Architecture at the University of Bath.

Ways to deal with discrete data

P.W. Hawkes

Digital Image Processing Techniques.

Edited by Michael P. Ekstrom.
*Academic: 1984. Pp.372.
\$49.50, £40.*

Applications of Walsh and Related Functions: With an Introduction to Sequency Theory.

By K.G. Beauchamp.
Academic: 1985. Pp.308. \$55, £33.50.

MANY books have now been published on image processing, ranging from full-scale treatises to multi-author texts on certain specialized topics. Ekstrom's volume repeats much material already presented in more detail elsewhere, but it aims to meet a need not yet specifically catered for: how can an outsider know which of the many branches of image processing he ought to study in depth to solve his particular problem, and what hardware and software is already available? This is indeed a problem, for the introductory volumes are thick and the advanced ones incomprehensible to the uninitiated.

Ekstrom has invited a distinguished team to contribute short accounts of most of the main themes of image processing, from which the reader can rapidly grasp what the methods are capable of doing and whether they might be of use to him. The chapters cover enhancement, restoration, detection and estimation, 3-D reconstruction, coding, spectral estimation and analysis, and a longer concluding contribution surveys architecture, display hardware, software and

the various systems. This should therefore be regarded as a guidebook rather than a textbook, and as such it is extremely successful.

Image processing is the subject of one of the chapters in Beauchamp's new book on discrete functions, which conveniently brings together much scattered material, but which, owing no doubt to this diversity, is very uneven. The opening chapters on the functions and transforms themselves are clear and full, including many interesting details about such important questions as the problem of shift-variance and the conversion from one transform to another.

The second half of the book, in which applications as various as seismology, medical signal processing, image enhancement and restoration, non-sinusoidal electromagnetic radiation and minimization of logic functions are discussed in 120 pages, is much less satisfactory. In some cases the text provides a good introduction to the more detailed literature, but in others the treatment is so inadequate that omission of the topic would have been the better course. Section 6.3.3 on image restoration occupies barely one page, for example, and, still in Section 6.3, it is not much use to be told that "optical... images [are] always positive quantities and therefore do not obey completely normal statistical operations". Conversely, the four pages on nonlinear applications of signal processing do succeed in conveying the originality of Walsh functions. This, then, is a useful book, but one which could have been much better. □

P.W. Hawkes is Maître de Recherches at the Laboratoire d'Optique Electronique du CNRS, Toulouse.

Princeton

The Amphibian Ear

Ernest Glen Wever

This comprehensive work on the amphibian ear continues the examination of the nature and evolutionary development of the vertebrate ear begun in Ernest Glen Wever's earlier book, *The Reptile Ear*. Professor Wever studies the structure of the ear and its functioning as a receptor of sounds in all amphibian species [139] for which living representatives could be obtained. Structure is revealed by gross dissection methods and the microscopic examination of serial sections; functioning is shown by general behavioral observations, particularly by the recording of electrical potentials produced by the auditory hair cells in response to sounds. \$65.00

Now Available in Paperback

A Geological Miscellany

Compiled by G. Y. Craig and E. J. Jones

This book is the finest piece of light reading in geology I have ever encountered! ... I recommend *A Geological Miscellany* without reservation as a book that will bring both enlightenment and enjoyment."

—Ellis L. Yochelson, *Geology*
\$7.95 Not for sale in the British Commonwealth, except Canada

Colour

Why the World Isn't Grey
Hazel Rossotti

Why do pebbles look brighter when wet? Is there a "right" order in which to arrange a set of colored crayons? Why do some clothes change color when ironed? What are the colors you see when you press your eyes? To answer these and other questions, Hazel Rossotti uses scientific basics — matter, energy and eye structure — to discuss the colors of the natural world, the mechanism of color vision, and a range of color technology from ceramics to television. P: \$8.95. C: \$32.50

At your bookstore or

Princeton University Press

41 William Street, Princeton, NJ 08540

Vision as a guide to action

Stuart Sutherland

Visual Perception: Physiology, Psychology and Ecology.

By Vicki Bruce and Patrick Green.

Lawrence Erlbaum: 1985. Pp. 369. Hbk £26.95, \$39.95; pbk £8.95, \$14.95.

IN THE edifice of visual psychology are many mansions. Most textbooks on the subject describe only the more conventional ones — colour vision, the visual illusions, visual after-effects, flicker, perspective, adaptation to wearing prisms and so on. In *Visual Perception* Vicki Bruce and Patrick Green ignore these hoary topics and present instead some of the exciting new developments that have taken place over the past 20 years. Their book is unusual: it provides almost the first elementary account of the use of computer simulation to further our understanding of vision (rightly relying mainly on the work of David Marr); it devotes many pages to vision in insects; and rather than concentrating on how visual sensations are produced or visual recognition achieved, it examines in some detail how vision can guide action.

For good measure, the authors throw in a useful and up-to-date account of the neurophysiology of the visual system including a description of X, Y and W cells, of whose elusive properties nobody has yet succeeded in making sense. They write and argue clearly, and, as a supplement to existing textbooks or as an introduction to an advanced course on vision, the book will be invaluable; it could also be helpful to workers in other scientific disciplines who want to learn about recent developments in vision.

There are, however, a few faults, some of which arise because topics are not dealt with in sufficient breadth. For example, in describing how lines and edges are extracted from the image to achieve Marr's "primal sketch", Bruce and Green should have attempted to motivate the reader by giving the reasons why this is, rather surprisingly, a very difficult thing to do; or again, they describe Marr and Nishihara's method of representing objects for the purpose of recognition without attempting to evaluate carefully how far it fits in with our current knowledge of the visual system; and their account of scene analysis is so condensed that the uninformed reader will have difficulty understanding what it is all about. Moreover, several of their diagrams are completely wrong (for example those on pages 76, 131 and 264) and will puzzle or mislead the unsophisticated.

A more debatable feature is the attention paid to the ideas of J.J. Gibson, who believed that the dynamic visual image contained invariants that specified what was

present in the environment, and that these invariants were "picked up" directly by the eye with no underlying computational or inferential processes. The view is so silly that it is not worth taking seriously. Gibson was of course correct in believing that the starting point for what we see is the visual image, but who has ever doubted that? One example of an invariant is horizontal disparity between corresponding parts of the image on the left and the right eye: this disparity yields stereoscopic depth. But when we examine how disparity is actually detected and used, it turns out to be extremely complicated; despite much computational work on stereopsis over the past few years, there is still no theory that is completely adequate.

The same is true of most other invariants. Nor does one have to have read Gibson to understand that the time at which an object approaching at a constant speed will collide with someone's head can be derived from the expression dt/ds , where s is the angle subtended at the eye by any two points on the object, and t is the time of observation. As the authors point out, a more elaborate expression yields the hit-time for an object that is accelerating at a constant rate. Both the insect and the human brain almost certainly make use of formulae like these, the former for landing, the latter, among other things, for driving across busy intersections without collision. The use that organisms make of such invariants is fascinating and is spelt out in detail in the present book, but the formulations owe little or nothing to Gibson's ideas (which, after wasting much space on them, the authors are in any case eventually led to reject).

This misplaced emphasis on Gibson apart, Bruce and Green have written an interesting and pleasingly idiosyncratic book. If you want to know why sheep dogs can be trained to round up sheep, you will find the answer here, just as you will find answers to many other rarely discussed questions. And it is salutary to have a textbook that stresses that vision is a guide to action and not merely a device for providing a picture of the world in one's head. □

Stuart Sutherland is Director of the Centre for Research on Perception and Cognition at the University of Sussex.

New in paperback

● *Order out of Chaos: Man's New Dialogue with Nature* by Ilya Prigogine and Isabelle Stengers. Publisher is Flamingo (an imprint of Fontana), price is £3.95. For review see *Nature* 310, 341 (1984).

● *The Logic of Perception* by Irvin Rock. Publisher is MIT Press, price is \$11, £11.50. For review see *Nature* 311, 283 (1984).

● *The Left Hand of Creation: The Origin and Evolution of the Expanding Universe* by John D. Barrow and Joseph Silk. Publisher is Counterpoint (Unwin Paperbacks), price is £3.95. For review see *Nature* 307, 394 (1984).

Chemistry

Modern Crystallography III. Crystal Growth. By A.A. CHERNOV. Springer-Verlag: 1984. Pp. 517. ISBN 3-540-11516-1/0-387-11516-1. DM 154, \$56.10.

Structure and Bonding Vol. 57. M.J. CLARKE, J.B. GOODENOUGH, J.A. IBERS, C.K. JØRGENSEN, D.M.P. MINGOS, D. REINEN, P.J. SALDER and R.J.P. WILLIAMS (eds). Springer-Verlag: 1984. Pp. 199. ISBN 3-540-13411-5. DM 110, \$43.20.

Trace Analysis, Vol. 3. JAMES F. LAWRENCE (ed.). Academic: 1984. Pp. 258. ISBN 0-12-682103-8. \$49.50, £35.

Technology

Ceramics in Advanced Energy Technologies. H. KROCKEL, M. MERZ and O. VAN DER BIEST (eds). Reidel: 1984. Pp. 541. ISBN 90-277-1787-7. Dfl. 195, \$79.50.

Deformation of Ceramic Materials II. Materials Science Research, Vol. 18. RICHARD E. TRESSLER and RICHARD C. BRADT (eds). Plenum: 1984. Pp. 751. ISBN 0-306-41719-7. \$95.

Electron Spectroscopy: Theory, Techniques and Applications, Vol. 5. C.R. BRUNDLE and A.D. BAKER (eds). Academic: 1984. Pp. 378. ISBN 0-12-137805-5. \$75, £50.

Hybrid Microelectronic Technology. Electro-component Science Monographs Vol. 4. PETER MORAN (ed.). Gordon and Breach: 1984. Pp. 222. ISBN 0-677-06560-4. \$60.

Innovations in Biotechnology. ERICH H. HOUWINK and ROBERT R. VAN DER MEER (eds). Elsevier: 1984. Pp. 529. ISBN 0-444-42275-7. \$94.25, Dfl. 245.

Ion Implantation: Science and Technology. J.F. ZIEGLER (ed.). Academic: 1984. Pp. 635. ISBN 0-12-780820-2. \$55, £38.50.

Metal Cutting Principles. By MILTON C. SHAW. Oxford University Press: 1984. Pp. 594. ISBN 0-19-859002-4. £60.

Modelling in the Technology of Wastewater Treatment. By IMRE HORVATH. Pergamon: 1984. Pp. 202. ISBN 0-08-023978-1. £24.25, \$40.

Novel Application of Anomalous (Resonance) X-ray Scattering for Structural Characterization of Disordered Materials. By YOSHIO WASEDA. Springer-Verlag: 1984. Pp. 183. Pbk ISBN 3-540-13359-3/0-387-13359-3. Pbk DM 23, \$8.40.

Quantitative Electron Microscopy. J.N. CHAPMAN and A.J. CRAVEN (eds). Scottish Universities Summer School in Physics: 1984. Pp. 445. ISBN 0-905945-08-5. £30.

Research Techniques in Nondestructive Testing, Vol. VII. R.S. SHARPE (ed.). Academic: 1984. Pp. 376. ISBN 0-12-639057-6. \$80, £49.50.

Robotics Research. MICHAEL BRADY and RICHARD PAUL (eds). MIT Press: 1984. Pp. 1001. ISBN 0-262-02207-9. £57.

The Robotics Revolution: The Complete Guide for Managers and Engineers. By PETER B. SCOTT. Basil Blackwell: 1984. Pp. 345. ISBN 0-631-13162-0. Np.

Sci-Tech Report. JON TURNEY (ed.). Pluto: 1984. Pp. 386. Pbk ISBN 0-86104-761-3. Pbk £8.95.

Technology of Chemicals and Materials for Electronics. E.R. HOWELLS (ed.). Ellis Horwood: 1984. Pp. 333. ISBN 0-85312-771-9. £39.50.

Earth Sciences

Advances in Petroleum Geochemistry, Vol. 1. JIM BROOKS and DIETRICH WELTE (eds). Academic: 1984. Pp. 344. ISBN 0-12-032001-0. \$49.50, £35.10.

The Climate of Europe: Past, Present and Future. By HERMANN FLOHN and ROBERTO FANTECHI. Reidel: 1984. Pp. 356. ISBN 90-277-1745-1. Dfl. 130, \$49.

Climatic Changes on a Yearly to Millennial Basis. N.A. MÖRNER and W. KARLÉN (eds). Reidel: 1984. Pp. 667. ISBN 90-277-1779-6. Np.

A Description of Devices Used in the Study of Wind Erosion of Soils. By A.P. BOCHAROV. Balkema: 1984. Pp. 90. ISBN 90-6191-426-4. £9.50.

Developments and Applications of Geomorphology. JOHN E. COSTA and P. JAY FLEISHER (eds). Springer-Verlag: 1984. Pp. 372. ISBN 3-540-13457-3/0-387-13457-3. DM 118, \$43.

Fossils and Climate. P.J. BRENCHEY (ed.). Wiley: 1984. Pp. 352. ISBN 0-471-90418-X. £27.

Geography Matters. DOREEN MASSEY and JOHN ALLEN (eds). Cambridge University Press: 1984. Pp. 204. Hbk ISBN 0-521-26887-7; pbk ISBN 0-521-31708-8. Hbk £20, \$34.50; pbk £5.95.

A Geology for Engineers, 7th Edn. By F.G.H. BLYTH and M.H. DE FREITAS. Edward Arnold: 1984. Pp. 325. Pbk ISBN 0-7131-2882-8. Pbk £16.50.

Geophysical Data Analysis: Discrete Inverse Theory. By WILLIAM MENKE. Academic: 1984. Pp. 260. ISBN 0-12-490920-5. \$42.50, £30.

The Glaciers of Equatorial East Africa. By STEFAN HASTENRATH. Reidel: 1984. Pp. 353. ISBN 90-277-1572-6. Dfl. 195, \$72.50.

Introduction to Statistics for Geographers and Earth Scientists. By R.B.G. WILLIAMS. Macmillan, London: 1984. Pp. 349. Pbk ISBN 0-333-35275-0. Pbk £7.95.

The Nature of the Environment: An Advanced Physical Geography. By ANDREW GOUDIE. Basil Blackwell: 1984. Pp. 331. Pbk ISBN 0-631-13144-2. Pbk £7.50.

Neuvième Congrès International de Stratigraphie et de Géologie du Carbonifère. PATRICK K. SUTHERLAND and WALTER L. MANGER (eds). Southern Illinois University Press: 1984. Pp. 630. ISBN 0-8093-1169-0. \$75.

Paleozoic Salt Bearing Formations of the World. By MICHAEL A. ZHARKOV. Springer-Verlag: 1984. Pp. 427. ISBN 3-540-13133-7/0-387-13133-7. DM 160, \$62.10.

Pollutants in Porous Media: The Unsaturated Zone Between Soil Surface and Groundwater. B. YARON, G. DAGAN and J. GOLDSCHMID (eds). Springer-Verlag: 1984. Pp. 296. ISBN 3-540-13179-5/0-387-13179-5. DM 139, \$50.60.

Principles of Sedimentary Basin Analysis. By ANDREW D. MIALL. Springer-Verlag: 1984. Pp. 490. ISBN 0-387-90941-9/0-540-90941-9. DM 120, \$44.80.

The Surface Chemistry of Soils. By GARRISON SPOSITO. Oxford University Press: 1984. Pp. 234. ISBN 0-19-503421-X. £35.

Environmental Sciences

Greenhouse Effect and Sea Level Rise: A Challenge for this Generation. MICHAEL C. BARTH and JAMES G. TITUS (eds). Van Nostrand Reinhold: 1984. Pp. 325. ISBN 0-442-20991-6. \$24.50.

Remote Sensing for the Control of Marine Pollution. JEAN-MARIE MASSIN (ed.). Plenum: 1984. Pp. 466. ISBN 0-306-41734-0. \$72.50.

The Roots of Modern Environmentalism. By DAVID PEPPER. Croom Helm: 1984. Pp. 246. ISBN 0-7099-2064-4. £17.95.

Biological Sciences

The Adenoviruses. HAROLD S. GINSBERG (ed.). Plenum: 1984. Pp. 605. ISBN 0-306-41592-5. \$85.

Allergy to Chemicals and Organic Substances in the Workplace. By G.W. CAMBRIDGE and B.F.J. GOODWIN. Science Reviews, 28, High Ash Drive, Leeds LS17 8RA, UK: 1984. Pp. 67. Pbk ISBN 0-905927-66-4. Pbk £15, \$22.

Amino-Acids Peptides and Proteins, Vol. 15. J.H. JONES (ed.). Royal Society of Chemistry: 1984. Pp. 464. ISBN 0-85186-134-2. £78, \$140.

Assembly of Enveloped RNA Viruses. By MONIQUE DUBOIS-DALCO, KATHRYN V. HOLMES and BERNARD RENTIER. Springer-Verlag: 1984. Pp. 235. ISBN 3-211-81802-2/0-387-81802-2. DM 138.

Atlas of Butterflies in Britain and Ireland. By J. HEATH, E. POLLARD and J.A. THOMAS. Viking: 1984. Pp. 158. ISBN 0-670-80006-6. £17.95.

Bacterial and Viral Inhibition and Modulation of Host Defences. G. FALCONE et al. (eds). Academic: 1984. Pp. 249. ISBN 0-12-247980-7. \$24, £16.

The Barbary Macaque: A Case Study in Conservation. JOHN E. FA (ed.). Plenum: 1984. Pp. 369. ISBN 0-306-41733-2. \$49.50.

Biochemical Actions of Hormones. GERALD LITWACK (ed.). Academic: 1984. Pp. 392. ISBN 0-12-452811-2. \$58.

Biological Magnetic Resonance, 6. LAWRENCE J. BERLINER and JACQUES REUBEN (eds). Plenum: 1984. Pp. 300. ISBN 0-306-41683-2. \$47.50.

Biological Science, Energy and Environment. R. SOPER (ed.). Cambridge University Press: 1984. Pp. 472. Pbk ISBN 0-521-28407-4. Pbk £8.95.

The Biology of Ground-Dwelling Squirrels. JAN O. MURIE and GAIL R. MICHENER (eds). University of Nebraska Press: 1984. Pp. 459. ISBN 0-8032-3090-7. £24.65.

The Biology of Idiotypes. MARK I. GREENE and ALFRED NISONOFF (eds). Plenum: 1984. Pp. 507. ISBN 0-306-41646-8. Np.

Biomembranes. Membrane Fluidity. MORRIS KATES and LIONEL A. MANSON (eds). Plenum: 1984. Pp. 693. ISBN 0-306-41548-8. \$85.

The Biosynthesis and Metabolism of Plant Hormones. ALAN CROZIER and JOHN R. HILLMAN (eds). Cambridge University Press: 1984. Pp. 288. ISBN 0-521-26424-3. £17.50.

Bone Marrow Biopsies Revisited. By R. BARTL, B. FRISCH and R. BURKHARDT. Karger: 1984. Pp. 138. ISBN 3-8055-3937-1. DM 69, \$34.75.

Cariology Today. B. GUGGUNHEIM (ed.). Karger: 1984. Pp. 396. ISBN 3-8055-3761-1. DM 196, \$98.25.

Cattle Diseases. By H.G. BELSCHNER. Angus & Robertson: 1984. Pp. 378. ISBN 0-207-14823-6. £17.50.

Cell Surface Dynamics: Concepts and Models. ALAN S. PERELSON, CHARLES DELISI and FREDERIK W. WIEGEL (eds). Dekker: 1984. Pp. 550. ISBN 0-8247-7115-X. \$85.

Cellular and Molecular Biology of Neoplasia. TAK W. MAK and IAN TANNOCK (eds). Alan R. Liss: 1984. Pp. 230. ISBN 0-8451-0236-2. £44.

The Centipedes and Millipedes of Southern Africa. By R.F. LAWRENCE. Balkema: 1984. Pp. 148. ISBN 0-86961-142-9. £15.

Cephalopod Life Cycles, Vol. 1 Species Accounts. P.R. BOYLE (ed.). Academic: 1984. Pp. 475. ISBN 0-12-123001-5. \$120, £70.

The Cereal Rusts. Vol.1 Origins, Specificity, Structure, and Physiology. WILLIAM R. BUSHNELL and ALAN P. FOELFS (eds). Academic: 1984. Pp. 546. ISBN 0-12-148401-7. \$65, £50.50.

Cerebral Ischemia. ANDRE BES et al. (eds). Elsevier Biomedical: 1984. Pp. 546. ISBN 0-444-80586-9. \$78.75, Dfl. 205.

Chloroplast Metabolism: The Structure and Function of Chloroplasts in Green Leaf Cells, Revised Edn. By BARRY HALLIWELL. Oxford University Press: 1984. Pp. 259. Pbk ISBN 0-19-854585-1. Pbk £9.95.

Chromosomal Variation in Man: A Catalog of Chromosomal Variants and Anomalies, 4th Edn. By DIGAMBER S. BORGAONKAR. Alan R. Liss: 1984. Pp. 1002. ISBN 0-8451-0231-1. £72.

Chromosomes Today, Vol. 8. M.D. BENNETT, A. GROPP and U. WOLF (eds). George Allen & Unwin: 1984. Pp. 370. ISBN 0-04-575023-8. £30, \$50.

Cladistics: Perspectives on the Reconstruction of Evolutionary History. THOMAS DUNCAN and TOD F. STUESSY (eds). Columbia University Press: 1984. Pp. 312. ISBN 0-231-05430-0. \$45.50.

Colour Identification Guide to Butterflies of the British Isles. By T.G. HOWARTH. Viking: 1984. Pp. 151. ISBN 0-670-80355-3. £14.95.

Colour Identification Guide to Moths of the British Isles. By BERNARD SKINNER. Viking: 1984. Pp. 267. ISBN 0-670-80354-5. £20.

Comparative Biochemistry and Physiology of Enzymatic Digestion. By H.J. WONK and J.R.H. WESTERN. Academic: 1984. Pp. 501. \$80, £55.

Current Concepts in Plant Taxonomy. V.H. HEYWOOD and D.M. MOORE (eds). Academic: 1984. Pp. 432. ISBN 0-12-347060-9. \$50, £35.

Current Topics in Bioenergetics, Vol. 13. C.P. LEE (ed.). Academic: 1984. Pp. 283. ISBN 0-12-152513-9. \$55, £42.50.

Current Topics in Cellular Regulation. BERNARD L. HORECKER and EARL R. STADTMAN (eds). *Academic*:1984. Pp.268. ISBN 0-12-152823-5. \$41, £32.

Current Topics in Developmental Biology, Vol. 19. Palate Development: Normal and Abnormal Cellular and Molecular Aspects. ERNEST F. ZIMMERMAN (ed.). *Academic*:1984. Pp.251. ISBN 0-12-153119-8. \$48, £34.

Diseases of the Hair and the Scalp. Current Problems in Dermatology, Vol. 12. J.W.H. MALI (ed.). *Karger*:1984. Pp.252. ISBN 3-8055-3783-2. DM 98, \$49.25.

Early Brain Damage, Vol. 2. Neurobiology and Behavior. STANLEY FINGER and C. ROBERT ALMLI (eds). *Academic*:1984. Pp.387. ISBN 0-12-052902-5. \$49.50, £35.

Ecological Effects of Fire in South African Ecosystems. PETER DE V. BOOYSEN and NEIL M. TANTON (eds). *Springer-Verlag*:1984. Pp.426. ISBN 3-540-13501-4/0-387-13501-4. DM 79, \$31.

The Ecology and Physiology of the Fungal Mycelium. D.H. JENNINGS and A.D.M. RAYNER (eds). *Cambridge University Press*:1984. Pp.564. ISBN 0-521-25413-2. £57.50.

Endocrine Correlates of Reproduction. KYOICHIRO OCHIAI *et al.* (eds). *Springer-Verlag*: 1984. Pp.320. ISBN 3-540-13514-6/0-387-13514-6. DM 96, \$35.

Enzyme Catalysis and Control. MARLENE DELUCA, HERNY LARDY and RICHARD L. CORSS (eds). *Academic*: 1984. Pp.509. ISBN 0-12-152824-3. \$89, £62.50.

Enzyme Structure and Mechanism, 2nd Edn. By ALAN FERSHT. *W.H. Freeman*:1984. Pp.475. Hbk ISBN 0-716-1614-3; pbk ISBN 0-7167-1615-1. Hbk £28.95; pbk £14.95.

Essentials of Physiology, 2nd Edn. By J.F. LAMB *et al.* *Blackwell Scientific*:1984. Pp.465. Pbk ISBN 0-632-01259-5. Pbk £12.50.

Eutrophication and Land Use. By WILLIAM M. LEWIS *et al.* *Springer-Verlag*:1984. Pp.202. ISBN 0-387-90961-3/3-540-90961-3. DM 118, \$46.30.

Applied Biological Sciences

Chemical Regulation of Immunity in Veterinary Medicine. MEIR KENDE, JOSEPH GAINER and MICHAEL CHIRIGOS (eds). *Alan R. Liss*: 1984. Pp.620. ISBN 0-8451-5011-1. £67.

Current Treatment of Anorexia Nervosa and Bulimia. By PAULINE S. POWERS and ROBERT C. FERNANDEZ (eds). *Karger*: 1984. Pp.348. ISBN 3-8055-3879-0. DM 117, \$58.75.

Early Brain Damage, Vol. 1 Research Orientations and Clinical Observations. C. ROBERT ALMLI and STANLEY FINGER (eds). *Academic*: 1984. Pp.368. ISBN 0-12-052901-7. \$49.50, £35.

Electrophysiology of Epilepsy. By P.A. SCHWARTZKROIN and H. WHEAL. *Academic*: 1984. Pp.420. ISBN 0-12-633080-8. \$69, £42.

Energy Intake and Activity. Current Topics in Nutrition and Disease (Vol.11). ERNESTO POLLITT and PEGGY AMANTE (eds). *Alan R. Liss*: 1984. Pp.430. ISBN 0-8451-1610-X. £44.00.

Guide to Clinical Studies and Developing Protocols. By BERT SPILKER. *Raven*: 1984. Pp.319. ISBN 0-88167-018-9. \$53.50.

Health Hazards of Milk. D.L.J. FREED (ed.). *Bailliere Tindall*: 1984. Pp.281. ISBN 0-7020-1049-9. £14.95.

Hybridoma Technology in Agricultural and Veterinary Research. NORMAN J. STERN and H. RAY GAMBLE (eds). *Rowman & Allanheld*, 81 Adams Drive, Totowa, NJ 07512: 1984. Pp.338. ISBN 0-8476-7362-6. \$48.50.

Instrument Evaluation in Biomedical Sciences. By JAMES L. DRISCOLL, BENJAMIN J. GUDZINOWICZ and HORACE F. MARTIN. *Dekker*: 1984. Pp.328. ISBN 0-8247-7184-2. \$59.75.

Interpretation of Breast Biopsies. By DARRYL CARTER. *Raven*: 1984. Pp.211. ISBN 0-88167-022-7. \$44.50.

Introduction to Laboratory Hematology and Hematopathology. By HAROLD R. SCHUMACHER, DAVID F. GARVIN and DOUGLAS A. TRIPLETT. *Alan R. Liss*: 1984. Pp.624. ISBN 0-8451-0235-4. £37.50.

Isotopes and Radiation in Agricultural Sciences. Vol. 2 Animals, Plants, Food and the Environment. M.F. L'ANNUNZIATA and J.O. LEGG (eds). *Academic*: 1984. Pp.356. ISBN 0-12-436602-3. \$75, £49.

Matters of Life and Death: Risks vs. Benefits of Medical Care. By EUGENE D. ROBIN *W.H. Freeman*: 1984. Pp.205. Pbk ISBN 0-7167-1681-X. Np.

Medical Images and Displays. By R. STUART MACKAY. *Wiley*: 1984. Pp.276. ISBN 0-471-89617-9. £49.80.

Modern Methods in Pharmacology Vol. 2. SYDNEY SPECTOR and NATHAN BACK (eds). *Alan R. Liss*: 1984. Pp.140. ISBN 0-8451-2501-X. £22.00.

Neuro-Otology and Skull Base Surgery. P. VAN DEN BROEK *et al.* (eds). *Karger*: 1984. Pp.280. ISBN 3-8055-3887-1. DM 196, \$98.25.

Nutrition and Cancer. By RAYMOND J. SHAMBERGER. *Plenum*: 1984. Pp.372. ISBN 0-306-41553-4. \$49.50.

Nutritional and Toxicological Aspects of Food Safety. MENDAL FRIEDMAN (ed.). *Plenum*: 1984. Pp.584. ISBN 0-306-41708-1. \$79.50.

Oligonucleotide Synthesis: A Practical Approach. M.J. GAIT (ed.). *IRL*: 1984. Pp.232. Pbk ISBN 0-904147-74-6. Pbk £11, \$20.

Pain Treatment: Pituitary Neuroadenolysis in the Treatment of Cancer Pain and Hormone-dependent Tumours. STEFANO ISCHIA, SAMPSON LIPTON and GIANFRANCO MAFFEZZOLI (eds). *Raven*: 1984. Pp.119. ISBN 88-85037-44-5. \$44.50.

Pharmacokinetics: A Modern View. LESLIE Z. BENET, GERHARD LEVY and BOBBE L. FERRAILOL (eds). *Plenum*: 1984. Pp.531. ISBN 0-306-41810-X. Np.

Phosphate and Mineral Metabolism. Advances in Experimental Medicine and Biology, Vol. 178. SHAUN G. MASSRY, GIUSEPPE MASCHIO and EBERHARD RITZ (eds). *Plenum*: 1984. Pp.485. ISBN 0-306-41731-6. \$69.50.

Progress and Controversies in Oncological Urology. KARL H. KURTH *et al.* (eds). *Alan R. Liss*: 1984. Pp.608. ISBN 0-8451-5003-0. Np.

Progress in Drug Metabolism. J.W. BRIDGES and L.F. CHASSEAUD (eds). *Taylor & Francis*: 1984. Pp.407. ISBN 0-85066-269-9. £35.

Renal Effects of Petroleum Hydrocarbons, Vol. VII. By MYRON A. MEHLMAN *et al.* (eds). *Princeton*: 1984. Pp.302. ISBN 0-911131-00-0. Np.

Research in Mental Illness. By DEREK RICHTER. *Heinemann*: 1984. Pp.181. Pbk ISBN 0-433-27601-0. Pbk £4.95.

Role of Medroxyprogesterone in Endocrine-Related Tumors, Vol. 3. A. PELLEGRINI *et al.* (eds). *Raven*: 1984. Pp.191. ISBN 0-88167-032-4. \$41.50.

Second Cancer in Relation to Radiation Treatment for Cervical Cancer. N.E. DAY and J.D. BOICE (eds). *Oxford University Press*: 1984. Pp.207. ISBN 0-19-723052-0. £17.50.

Serotonin in Affective Disorders. J. MENDLEWICZ, B. SHOPSIN and H.M. VAN PRAAG (eds). *Karger*: 1984. Pp.90. ISBN 3-8055-3898-7. DM 65, \$32.50.

Staphylococci and Staphylococcal Infections. Vol. 1 Clinical and Epidemiological Aspects. C.S.F. EASMON and C. ADLAM (eds). *Academic*: 1984. Pp.384. ISBN 0-12-228101-2. \$65, £39.50.

Theory and Research in Behavioural Pediatrics. HIRAM E. FITZGERALD, BARRY M. LESTER and MICHAEL W. YOGMAN (eds). *Plenum*: 1984. Pp.283. ISBN 0-306-41566-6. Np.

Thrombosis. By M. VERSTRAETE and J. VERMYLEN. *Pergamon*: 1984. Pp.339. Pbk ISBN 0-08-031976-9. Pbk £12, \$19.50.

Viral Chemotherapy, Vol. 1. D. SHUGAR (ed.). *Pergamon*: 1984. Pp.573. ISBN 0-08-029821-4. Np.

Warble Fly Control in Europe. CHANTAL BOULARD and H. THORNBERRY (eds). *Balkema*: 1983. Pp.288. ISBN 90-6191-295-4. \$18.50, £11.

Psychology

Advances in Clinical Child Psychology. BENJAMIN B. LAHEY and ALAN E. KAZDIN (eds). *Plenum*: 1984. Pp.352. ISBN 0-306-41659-X. \$39.50.

Behavioral Theories and Treatment of Anxiety. SAMUEL M. TURNER (ed.). *Plenum*: 1984. Pp.437. ISBN 0-306-41593-3. \$45.

©1985Nature Publishing Group

Emergency Psychiatry: Concepts, Methods, and Practices. ELLEN L. BASSUK and ANN W. BIRK (eds). *Plenum*: 1984. Pp.445. ISBN 0-306-41655-7. \$45.

Mathematical Models of Attitude Change: Change in Single Attitudes and Cognitive Structure, Vol. 1. JOHN E. HUNTER, JEFFERY E. DANES and STANLEY H. COHEN (eds). *Academic*: 1984. Pp.339. ISBN 0-12-361901-7. \$59, £41.50.

Philosophy of Psychology. By JOSEPH MARGOLIS. *Prentice/Hall*: 1984. Pp.107. Pbk ISBN 0-13-664326-4. Pbk £12.30.

Physiological Correlates of Human Behaviour: Vol. 1 Basic Issues. ANTHONY GALE and JOHN A. EDWARDS (eds). *Academic*: 1983. Pp.350. ISBN 0-12-273901-9. \$39.50, £25.

Physiological Correlates of Human Behaviour: Vol. 3 Individual Differences and Psychopathology. ANTHONY GALE and JOHN A. EDWARDS (eds). *Academic*: 1984. Pp.298. ISBN 0-12-273903-5. \$48, £28.

A Primer of Psychobiology: Brain and Behavior, 2nd Edn. By TIMOTHY J. TEYLER. *W.H. Freeman*: 1984. Pp.181. Hbk ISBN 0-7167-1459-0; pbk ISBN 0-7167-1460-4. Hbk £17.95; pbk £8.95.

Psychological Testing. By JOHN R. GRAHAM and ROY S. LILLY. *Prentice/Hall*: 1984. Pp.433. ISBN 0-13-732652-1. £28.80.

The Psychology of Sexual Diversity. KEVIN HOWELLS (ed.). *Basil Blackwell*: 1984. Pp.270. ISBN 0-631-13669-X. £19.50.

The Spectrum of Psychiatric Research. MICHAEL SHEPHERD (ed.). *Cambridge University Press*: 1984. Pp.240. ISBN 0-521-26585-1. £20.

Theory in Psychopharmacology, Vol. 2. S.J. COOPER (ed.). *Academic*: 1984. Pp.247. ISBN 0-12-188002-8. \$45, £25.

Sociology

Children and Arson: America's Middle Class Nightmare. By WAYNE S. WOODEN and MARTHA LOU BERKEY. *Plenum*: 1984. Pp.267. ISBN 0-306-41773-1. \$16.95.

Determinants of Fertility in Developing Countries. Vol. 1 Supply and Demand for Children. RODOLFO A. BULATAO and RONALD D. LEE (eds). *Academic*: 1983. Pp.642. ISBN 0-12-140501-X. \$37.

Remarriage: A Family Affair. By LILLIAN MESSINGER. *Plenum*: 1984. ISBN 0-306-41770-7. \$16.95.

Anthropology

The Archaeology of Wetlands. By JOHN COLES. *Edinburgh University Press*: 1984. Pp.111. Pbk ISBN 0-85224-503-3. Pbk £5.50.

Southern African Prehistory and Paleoenvironments. RICHARD G. KLEIN (ed.). *Balkema*: 1984. Pp.404. ISBN 90-6191-097-8. £16.50.

The Trias and its Ammonoids: The Evolution of a Time Scale. By E.T. TOZER. *Geological Survey of Canada*: 1984. Pp.171. Pbk ISBN 0-660-11600-6. Np.

General

Adam and Evolution. By MICHAEL PITMAN. *Hutchinson*: 1984. Pp.268. ISBN 0-09-155390-3. £9.95.

Agreement and Anaphora: A Study of the Role of Pronouns in Syntax and Discourse. By PETER BOSCH. *Academic*: 1983. Pp.260. ISBN 0-12-118820-5. \$35.

Analysis of Messy Data, Vol. 1: Designed Experiments. By GEORGE A. MILLIKEN and DALLAS E. JOHNSON. *Van Nostrand Reinhold*: 1984. Pp.473. ISBN 0-534-02713-X. \$45.

The Analysis of Plastics. By T.R. CROMPTON. *Pergamon*: 1984. Pp.445. ISBN 0-08-026251-1. £29.50, \$49.50.

Analysis of Structural Learning. By M.A. JEEVES and G.B. GREER. *Academic*: 1984. Pp.269. ISBN 0-12-382080-4. \$40, £25.

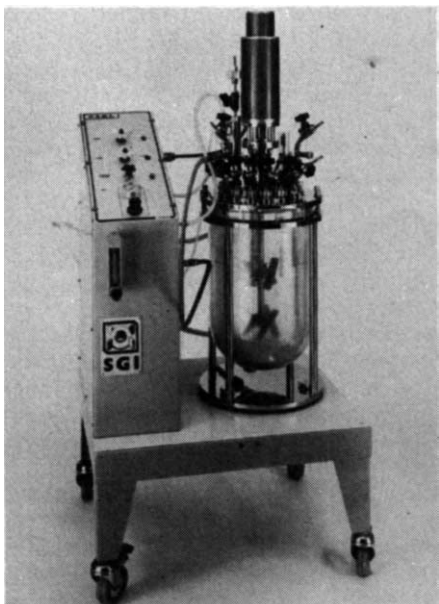
Annual Reports on Fermentation Processes, Vol. 7. GEORGE T. TSAO (ed.). *Academic*: 1984. Pp.358. Pbk ISBN 0-12-040307-2. \$42.50, £33.

Atoms of Silence. By HUBERT REEVES. *MIT Press*: 1984. Pp.244. ISBN 0-262-18112-6. £14.20.

Putting the technology into biology

Recent entrants onto the expanding 'biotechnology market' include sophisticated computerized systems for monitoring fermentations.

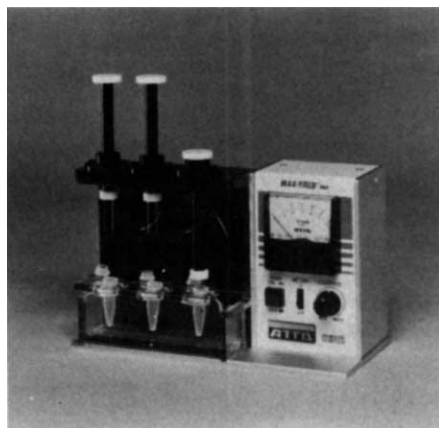
● A new all-glass bioreactor from Astra Scientific has been designed specifically for cell culture applications. Working volume is 10 litres (20 litres total), and the vessel has a hemispherical bottom and is dimensioned to provide the optimum 1:1 height to depth ratio required in this application. The vessel is completely jacketed up to its maximum working volume capacity for accurate temperature control. Two impellers provide gentle agitation, and accurate speed is assured through the use of a tachometer and feedback control loop.
Reader Service No. 100.



SGI's all-glass bioreactor is marketed by Astra Scientific in the United States.

● Monoclonal antibody scale-up is among the services offered to the biotechnologist by Celltech. Celltech specializes in bulk antibody production having the facilities to produce monoclonals in kilogram quantities. Celltech is also active in the business information field — Abstracts in BioCommerce is a commercial news index available as an on-line computer database.
Reader Service No. 101.

● The new Dako catalogue for 1985 permits easy location of any one of over 400 antisera, purified proteins and immunohistochemical staining kits — including monoclonal and polyclonal antibodies. Recent additions include a new line of fluorescein- and peroxidase-conjugated F(ab')₂ fragments of antibodies. A FITC-conjugated F(ab')₂ rabbit antibody against mouse immunoglobulins is useful in screening for immunoglobulin production in hybridoma cultures.
Reader Service No. 102.



For recovering DNA fragments from gels, the DNA-3 Max-Yield.

● The DNA-3 Max-Yield system, now available from Genetic Research Instrumentation, has been designed specifically to recover DNA fragments from electrophoresis gels quickly with high yields and no further nicking of the fragments. Up to 98% of each band fragment may be recovered and the system may be used as a purification procedure or for DNA recovery for further experimental assays. The DNA-3 will process up to three samples simultaneously and is complete with its own power supply, tube rack and microelectrodes. Gel fragments are placed within a special holder which in turn is placed within a 1.5 ml microcentrifuge tube. Between 200 and 500 µl of buffer is added to each tube and ice is added to the tube rack in which the tubes are resting. A pair of microelectrodes is lowered into each tube, one either side of the gel fragment, and voltage is applied. At a voltage setting of 20 volts, DNA will be removed from the fragments within 15 to 20 minutes.
Reader Service No. 103.

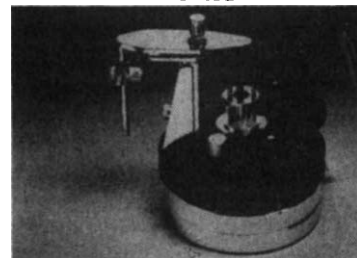
● A four-page bulletin describing Pall Biodyne ImmunoAffinity Membrane, a solid support for immobilization of antibodies and antigens, with particular application for use in immunodiagnostic test procedures, is available from the Biotechnology Division of Pall Ultrafine Filtration Corporation. Suggested protocols are provided for immobilization and binding of monoclonal or polyclonal antibodies and antigens to the membrane by wetting, immersion or perfusion. Protocols for the blocking of nonspecific protein binding to Biodyne ImmunoAffinity membranes are also discussed. The membrane is available in precut disks, squares, cartridges and roll form. It is offered in two standard pore sizes: 0.1 and 0.2 µm, but other pore sizes can be ordered.
Reader Service No. 104.

● Bacteriophage T7 gene 6 exonuclease, a 5'-3' exonuclease with a strong preference for double-stranded DNA, is now available from United States Biochemical Corporation. Its catalytic mechanism allows it to be used for controlled digestion of DNA in both analytical and preparative experiments. The exonuclease is available in packages of 2,000 or 10,000 units; each unit is enough enzyme to release 1 nmol of acid-soluble nucleotide in 15 min at 37°C.
Reader Service No. 105.

● Cross-reactivity with both human and mouse immunoglobulins has been virtually eliminated in Tago's expanded line of goat antibodies to rat IgG (H and L chains). Tago conjugated anti-rat IgG products are designed for use as a second antibody with rat monoclonal IgG in indirect immunoassays and histochemical staining. They are especially suitable for ELISA and flow cytometry. The range includes conjugates with fluorescein, lissamine rhodamine, horseradish peroxidase, alkaline phosphatase, β-galactosidase, or biotin.
Reader Service No. 106.

ADVERTISEMENTS

SINGER MICROMANIPULATOR MK III Patented



Perfect control in 3 Dimensions from a single joystick. Continuous variable reductions to suit all magnifications. No after sales maintenance needed.

Details from:
Singer Instrument Co. Ltd.,
Treborough Lodge Roadwater,
Watchet Somerset TA23 0QL
England. Tel. (0984) 40226 Telex:
337492 Comcab G.

Reader Service No.50

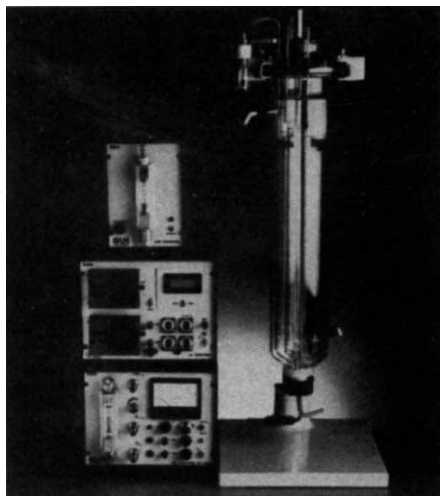
ERICH HARTH

WINDOWS ON THE MIND

Reflections on the
Physical Basis of
Consciousness

02.2503 X
288pp
£4.95

Reader Service No.49



The LH Fermentation 2-litre airlift fermenter.

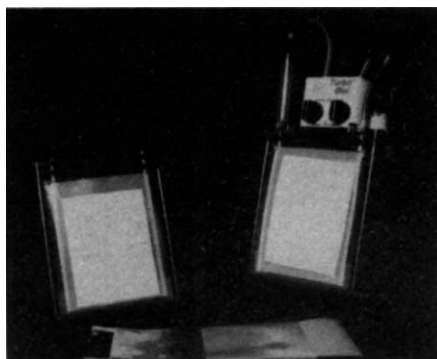
● A new 2-litre airlift fermenter that will be ideal for monoclonal antibody production, media optimization and other similar applications is available from LH Fermentation. Based on a 2-litre total volume glass vessel, the fermenter has a working volume of 1,700ml with a removable glass draught tube and a stainless-steel top plate. This has ports for oxygen and carbon dioxide electrodes and other sensors. Temperature control is via thermostatically controlled water circulated around a jacket on the glass vessel; agitation is achieved on the standard unit by the "air-lift" principle with a glass sinter providing the gas dispersion. Also from LH Fermentation is a new microprocessor-based fermentation control package which can monitor eight parameters in single or multiple fermentation. *Reader Service No. 107.*

● Four new restriction enzymes have been added to Boehringer Mannheim's line of premium enzymes — *Asp700*, *BanI*, *DdeI*, and *NcoI*. *Asp700* is an isoschizomer of *XmnI*. Another recent introduction from Boehringer is a ribonuclease inhibitor prepared to high quality for molecular biology. The preparation is supplied with a volume activity of 10–50 units per μl . It is checked for the absence of endonucleases and nicking activity.

Reader Service No. 108.

● Promega Biotec's Protoclone GT system allows the cloning and expression of a DNA insert in one day. Protoclone GT is a lambda gtl cloning and expression system which is particularly useful in the construction of cDNA libraries. It achieves high cloning efficiencies and a high percentage of insert-containing clones. And for RNA probe synthesis, Promega Biotec has introduced Riboprobe Gemini, a system allowing selective synthesis of RNA from either DNA strand following a single plasmid construction. Riboprobe Gemini produces useful plasmids for probe synthesis regardless of the insert orientation. The kit consists of: (1) two specially prepared RNA polymerases, bacteriophage SP6 polymerase and T7 polymerase; (2) two plasmids, each containing SP6 and T7 promoters flanking a multiple cloning site; (3) positive control transcription template.

Reader Service No. 109.



North, south or west, Turbo Blot makes blotting simpler.

● The American Bionuclear Turbo Blot filter processing system is the first device of its kind to handle the set-up and washing of Southern, Northern and Western blots. Hybridization and antibody binding reactions are quickly and safely performed, without need of a heat sealer, via a disposable pouch system. Probe reagents are conveniently added and removed from the pouch by means of a syringe. Blots are washed in minutes in a vacuum-wash system providing backgrounds typically lower than with manual processing.

Reader Service No. 110.

● New Plant Products, the first trading activity of Agricultural Genetics Company, has published a new leaflet describing their range of rhizobium inoculants for legumes. NPP produces a high quality product based on sedge peat containing strains of rhizobia specially selected for their nitrogen fixing ability from the extensive collection of rhizobia held at Rothamsted Experimental Station. The basic range covers all the legume crops grown in Europe and NPP can also supply inoculants for use in the tropics, and for the more unusual legumes used for reclamation work. Agricultural Genetics Company was set up to make use of the fruits of the Agricultural and Food Research Council's plant biotechnology research programme.

Reader Service No. 111.

● New from SysTec Inc. is 1/2 μm phosphoramidite synthesis software for the Microsyn-1450A automated DNA synthesizer. The Microsyn-1450A syringe delivery system permits the addition of just 7.8 mg of phosphoramidite base per synthesis cycle. The quantity of CPG support is also halved in this new synthesis program (approximately 20 mg per synthesis). The 1/2 μm software does not compromise either coupling reaction efficiency (98.5–99.5%) or cycle time (6.25–7.5 minutes). It is available for use with either *O*-methyl di-isopropyl or β -cyanoethyl di-isopropyl phosphoramidites.

Reader Service No. 112.

● Dianorm's Liposomat achieves the reproducible preparation of uniformly sized unilamellar liposomes (formed within just 10 minutes) with very high encapsulation efficiency for both hydrophilic and lipophilic agents. The liposome size is selectable between 25 and 600 nm diameter, with a lipid loss of less than 1%. Sample volume is normally 5–10 ml but can be up to 200 ml if the Liposomat is used with other apparatus. The Liposomat is especially suitable for studying drug/protein/cell—liposome interactions *in vitro* and *in vivo*, for reconstitution experiments, and for the transfer of genetic material in gene- and biotechnology.

Reader Service No. 113.

ADVERTISEMENT

In Immunology Quality is Innovation

Bio-Yeda is a world leader in cytoskeletal protein antisera, monoclonal antibodies, conjugates to human immunoglobulins and IgG subclasses, antisera to hormones and drugs, and much more.

Bio-Yeda: an internationally recognized source for immunochemicals of the highest quality.

bioyeda

Serving Science and Medicine

Bio-Yeda Ltd. Kiryat Weizmann, 76326 Rehovot, Israel. Tel: 08 470425, Tlx: 361930 MYED IL

bw



Monoclonals in cartoon form.

● A layman's explanation of monoclonal antibodies is now available on videocassette from Hybritech Europe. The video, entitled "The Monoclonal Antibody Revolution", presents in cartoon form (1) The fundamental principles of the immune reaction; (2) how antibodies can be used for medical purposes; (3) the difficulties encountered with "polyclonal antibodies"; (4) the breakthrough represented by monoclonal antibodies; (5) immediate medical applications; (6) near-term future potential.

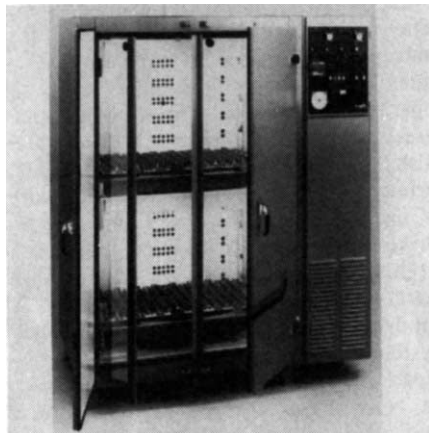
Reader Service No. 114.

● Two new monoclonal antibody purification systems (MAPS) utilizing affinity chromatography and hydroxylapatite (HPHT) have been introduced by Bio-Rad. Affi-Gel protein A MAPS makes use of a column matrix of protein A crosslinked to agarose beads. When used with specially optimized MAPS binding, elution and regeneration buffers, Affi-Gel protein A has a binding capacity for mouse IgG₁ from ascites fluid that it is 8-10 times higher than that attainable by published methods. Affi-Gel protein A is also ideal for purifying human immunoglobulins, with a binding capacity of 20 mg of purified IgG per ml of gel. HPHT-MAPS can be used in a dedicated hydroxylapatite HPLC system for separating monoclonal antibodies from usual contaminants, such as albumin and transferrin, as well as other contaminating immunoglobulins. Using the HPHT-MAPS system, mouse monoclonal antibodies can be ultra-purified regardless of class or sub-class.

Reader Service No. 115.

● Several new hybridization probe DNAs have been added to the Oncor range. These highly purified, sequence specific DNA fragments are designed for use in nick-translation reactions and for the detection of oncogenic sequences by nucleic acid hybridization. The new probes include DNAs to detect the following sequences: *abl*, *Alu*, *erb-1*, *fes*, *sis* and *src*. Also available are antibodies to *src* and epidermal growth factor receptors. The probes are supplied in a kit containing control DNAs. Technical data sheets are available on request from the UK distributors, Stratech Scientific Ltd in London (tel. 01 354 2601).

Reader Service No. 116.



Four individually-controlled shakers make up the Bioengineering Associates environmental control cabinet.

● Bioengineering Associates Inc. has introduced an environmental control cabinet containing four individually controlled shaking units. The flow of air guarantees uniform distribution of temperature, gaseous mixtures and humidity. Shaker heights are adjustable, allowing different shakers to be run with various size flasks; as well as a variety of speeds between 10 and 400 r.p.m. The temperature range is approximately 15°C below room temperature to 80°C with $\pm 0.2^\circ\text{C}$ accuracy. Magnetic drive ensures silent vibration-free running, without the need to anchor the four units.

Reader Service No. 117.

● Seraclone fetal calf serum is specially selected for its ability to support the growth of mouse hybridoma and/or myeloma cells in limiting dilution cloning in microtitre plates at 1 cell per well. Only those fetal calf sera which support cloning efficiencies of $\pm 100\%$ as predicted by Poisson distribution frequencies are given the label Seraclone. Available from Sera-Lab of Crawley Down in Sussex, this new grade of fetal calf serum is suitable for all phases of monoclonal antibody production.

Reader Service No. 118.

● The latest edition of the Serotec catalogue of monoclonal and polyclonal reagents has many new additions including sheep anti-human antisera to complement proteins, thyroid hormones, coagulation factors, serum proteases and inhibitors, conjugated to FITC, TRITC, peroxidase and alkaline phosphatase; purified monoclonal antibodies to immunoglobulins, serum components, MHC antigens and leukocytes; and mouse and rat monoclonal antibodies to MHC antigens and immunoglobulins. Serotec has established a network of distributors in Australia, Denmark, Greece, Ireland, Italy, Israel, Japan, New Zealand, Portugal, Spain, Sweden, Switzerland, Taiwan, The Netherlands, West Germany, Korea and Canada and is in final negotiations with companies in the United States.

Reader Service No. 119.

● Clontech now has cDNA libraries available for researchers. These libraries are prepared from human, rat and mouse tissues, from various cell lines, and there is also a yeast genomic library. The vectors are lambda gt10, gt11 and pBR322.

Reader Service No. 120.

● MicroGenie from Beckman Instruments is a software program which can collect, analyse and compare nucleic acid and protein sequences. MicroGenie can be used by the computer novice, guided with simple menus. It can analyse sequences of up to 60,000 nucleotides, enough for bacteriophage lambda or adenovirus. The memory stores up to a million nucleotides.

Reader Service No. 121.

ADVERTISEMENT

In Immunology Quality is Reliability

Bio-Yeda's consistently reliable reagents help scientists achieve exceptionally specific and reproducible results—time after time. Bio-Yeda: an internationally recognized source for immunochemicals of the highest quality.

bio.yeda

Serving Science and Medicine

Bio-Yeda Ltd. Kiryat Weizmann, 76326 Rehovot, Israel. Tel: 08 470425, Tlx: 361930 MIYED IL

by8

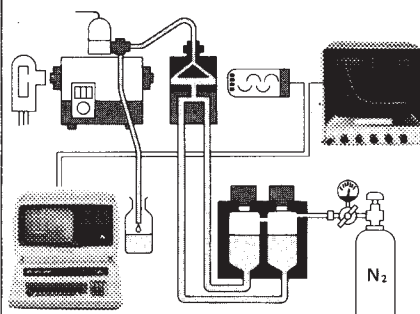
ADVERTISEMENT

UNION GIKEN

First choice for

STOPPED FLOW

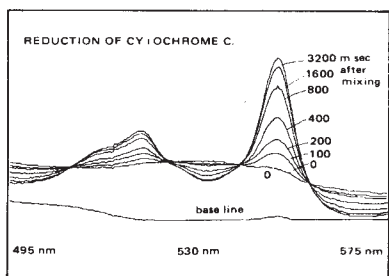
reaction analysis



Union Giken apparatus achieves a dead time as short as $\frac{1}{2}$ millisecond for measurement of fast reactions. Gas pressure is directly applied to the surface of the reactants in each reservoir, mixing and flow being controlled from the end of the system by a high-response stop valve. The RA-401 STOPPED FLOW SPECTROMETER is the ideal match for fast reactions.



The RA-451 DATA PROCESSOR generates a simulated reaction curve from the measured results and automatically calculates the rate constant.



The RA-415 RAPID SCAN ATTACHMENT monitors the change in absorption spectrum during the reaction.

Other attachments are available for T-jump, P-jump, fluorescence and flash photolysis.

Please visit us Halle 6 III, Stand KL 24 - 25, ACHEMA 85 (ISA).

Contact the sole export agent for more details:—

ATAGO BUSSAN CO., LTD.
7-23, 5-chome Shimbashi, Minato-ku,
Tokyo 105, Japan
Telex: 28421 Cable: TAGOA TOKYO
Phone: (03) 432-8741



Computer-directed DNA synthesis — Autogen 5500.

● Genetic Design of Watertown Massachusetts has developed the AutoGen 6500, the first automated DNA synthesizer to incorporate computer-directed variable-cycle control of the synthesizing process. The new system has the capacity for automatic synthesis of as many as three sequential 100-base fragments with no operator intervention. The AutoGen 6500 uses an Apple IIe computer (128KB, dual disks, CRT, voice synthesizer, Okidata 92 printer) for all control functions. Software is user-programmable by simple menu-driven commands. In addition, numerous utilities are provided for cycle and function parameterization defined by the user. A modem and printer are included with each system.

Reader Service No. 122.

● Now available in the UK from P & S Biochemicals of Liverpool, Promega Biotech's Packagene system is a single-extract *in vitro* lambda DNA packaging system. By combining all the necessary components and buffers in one tube, the system enables the user to conduct a packaging reaction simply by adding a sample of DNA to a volume of Packagene and incubating. Packaging efficiencies of over 10^8 PFU per μ g DNA are guaranteed, allowing effective and successful construction of DNA libraries.

Reader Service No. 123.



A new medium for hybridomas.

● Ventrex Laboratories have formulated a new serum-free cell culture medium, HL-1, that is ideal for hybridomas and lymphoid cells. HL-1 will facilitate the *in vitro* scale-up of monoclonal antibody production and simplifies or eliminates purification steps. In the United Kingdom, HL-1 is available from Tissue Culture Services of Slough.

Reader Service No. 124.

● A powerful new data acquisition and logging package has been introduced by the Laboratory Computer Systems Division of Milequip, the Gloucestershire based computer systems house. The package, known as Logger, is ideal for use with medium- or long-term biotechnological and process biochemical experiments and by technicians with no previous computer experience. The program runs in real time with a variable scanning interval. Following each scan, readings are displayed with an average facility for data reduction of a large number of scans, if required. Readings outside of preselected parameters sound an audible alarm, which may be deactivated if required.

Reader Service No. 125.

● The LSI mammalian cell transfection kit has been developed to make the transfer of stably expressing genes into mammalian cells a simple routine operation. Each kit includes: (1) All the pre-tested solutions for calcium phosphate transfecting up to 100 100-mm plates of cells; (2) a detailed protocol; (3) sterile 0.2- μ m syringe filters; (4) sterile storage vials; (5) test results for each lot. This new LSI kit is available from Vanguard International of Neptune, New Jersey.

Reader Service No. 126.

● Mentor is a new computer process control and data logging system for fermentation, developed by Life Science Laboratories Ltd of Luton. The software is optimized so that the Mentor system can be installed on an existing as well as a new fermentation plant without delay. Mentor 1 hardware is IBM PC based and utilizes a colour VDU, floppy disk drive, Winchester disk and six-pen colour plotter. The system is completely menu-driven with all aspects of configuration and operation being available to the keyboard user through a high-level language. Control sequences for operation of valves and loops can be specified by the user such that routines like sterilization, inoculation and transfers can be carried out automatically.

Reader Service No. 127.

● Bethesda Research Laboratories' new murine reverse transcriptase, Cloned M-MLV RT, synthesizes a higher percentage of full-length cDNA from mRNA than does avian reverse transcriptase. Murine RT is free of many of the contaminants common in avian RT preparations. BRL has cloned the reverse transcriptase coding sequence from M-MLV (Moloney murine leukaemia virus), ensuring reproducibly high performance. Full-length cDNAs can be synthesized more efficiently using Cloned M-MLV RT because it does not contain the DNA endonuclease activity associated with AMV RT. Purity is greater than 95% and there is no detectable DNase or RNase.

Reader Service No. 128.

These notes are based on information provided by the manufacturers. Use the reader service card bound inside the journal to obtain further information.